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FOOD PRODUCING ANIMALS

Criteria and Procedures for Evaluating Assays for Carcinogenic Residues

Title 21—Food and Drugs

CHAPTER I—FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

SUBCHAPTER A—GENERAL

SUBCHAPTER E—ANIMAL DRUGS, FEEDS, AND RELATED PRODUCTS

[Docket No. 77N-0026]

CHEMICAL COMPOUNDS IN FOOD-PRODUCING ANIMALS

Criteria and Procedures for Evaluating Assays for Carcinogenic Residues in Edible Products of Animals

The Food and Drug Administration (FDA) is establishing procedures and minimum criteria to ensure the absence of carcinogenic residues in edible products derived from food-producing animals that are administered drugs, food additives, or color additives. These regulations set forth below provide an operational definition of the no-residue requirement of the so-called "DES proviso" to the anticancer clauses, sections 409(c)(3)(A), 512(d)(1)(H), and 706(b)(5)(B), of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 348(c)(3)(A), 360b(d)(1)(H), and 376(b)(5)(B)). The regulations also establish criteria for acceptance of assay methods and procedures for establishing suitable post-administration withdrawal periods to prevent the occurrence of carcinogenic residues in edible products. The regulations shall become effective on March 21, 1977.

Prior to July 19, 1973, FDA had applied the proviso to the anticancer clauses of the act on a case-by-case basis, without published criteria. The Commissioner of Food and Drugs concluded that it was appropriate and necessary to establish such criteria and procedures for their application through rule making in order to permit public discussion of the scientific, legal, and policy issues involved. Accordingly, the Commissioner issued these regulations as a proposal, published in the FEDERAL REGISTER of July 19, 1973 (38 FR 19226), and afforded 60 days for public comment.

Forty-six comments on the proposal were received. These were submitted by scientists affiliated with consumer groups, universities, scientific societies, State and Federal agencies, trade associations, and affected manufacturers, and some from nonaffiliated individuals. Many comments revealed sharp divergence of opinion concerning FDA's interpretation of the proviso to the anticancer clauses of the act. For this reason, the Commissioner has set forth, initially, the legal and scientific rationale for these final regulations. Specific comments are described and discussed later in the preamble in connection with the provisions of the regulations to which they relate.

I. INTRODUCTION

A. STATUTORY BACKGROUND

Section 409 of the Federal Food, Drug, and Cosmetic Act establishes criteria and prescribes procedures for the approval of food additives that have been shown to be safe. As enacted in 1958, the anticancer (or so-called Delaney) clause of section 409 flatly proscribed the approval of any additive that "is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal * * *." As applied to additives added directly to human food, this language has remained unchanged. Accordingly, as a legal matter, section 409 precludes a finding by FDA that a direct food additive that has been shown to cause cancer in laboratory animals (or, of course, in man) can be safely added to food, in any amount, for any purpose. Section 706 of the act similarly prohibits the approval of any carcinogenic color additive.

The use of chemical compounds as additives to the feed of animals or as animal drugs has posed more complex problems. The act requires that compounds administered to animals as food additives, color additives, or animal drugs be shown to be safe for use. Under section 201(u) of the act (21 U.S.C. 321(u)), the term "safe" clearly embraces the health of man, as well as the health of the animals to which such compounds are given. Thus, in evaluating the safety compounds to be administered to animals raised or maintained for production of food for man, such as cattle, swine, and poultry, Congress has from the beginning recognized that consideration had to be given to the safety of possible residues of the compounds in the products of animals that become sources of food for man, i.e., meat, milk, and eggs.

Prior to 1962, the anticancer clauses in section 409 and section 706 did not distinguish between compounds added directly to human food and compounds that might indirectly enter human food through administration, as feed additives or drugs, to food-producing animals. The act was interpreted as forbidding FDA to approve the use of a carcinogenic animal drug whether or not the compounds might leave any residues in the edible tissues of the animal. However, Congress modified this flat prohibition in 1962 as part of the Drug Amendments of 1962, to focus on the likelihood that a compound would produce detectable residues. Section 409(c)(3)(A) now reads:

> * * * [N]o additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal, except that this proviso shall not apply with respect to the use of a substance as an ingredient of feed for animals which are raised for food production, if the Secretary finds (i) that, under the conditions of use and feeding specified in proposed labeling and reasonably certain to be followed in practice, such additive will not adversely affect the animals for which such feed is intended, and (ii) that no residue of the additive will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsections (f) and (g)) in any edible portion of such animal after slaughter or in any food yielded by or derived from the living animal * * *

Modification of the effect of the anticancer clause of section 409 was first suggested during congressional consideration of the Color Additive Amendments of 1960. In May 1960, the then-Secretary of Health, Education, and Welfare urged Congress to modify the act, explaining:

> There is * * * one respect to which the anticancer proviso has proved to be needlessly stringent as applied to the use of additives in animal feed. For example, in the case of various animals raised for food production, certain drugs are used in animal feed which will leave no residue in the animal after slaughter or in any food product (such as milk or eggs) obtained from the living animal, and which are therefore perfectly safe for man. If this is demonstrated with respect to any particular additive intended for animal feed, and the additive will not adversely affect the animal itself during its expected or intended life cycle, we can see no reason for not permitting such a use of an additive which could be highly useful and beneficial in the raising of animals for food * * *.
>
> We therefore have included in the enclosed draft bill an amendment to permit use of an additive in animal feed under the above-mentioned conditions.
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> * * * * *
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> [U]nder the amendment, the assay methods applicable in determining whether there will be a residue shall be those prescribed or approved by us by regulations. This will give reasonable certainty in that regard, although, of course, such regulations may from time to time be changed as new scientific developments demonstrate a need for change. It should be clearly understood that the industry still would have the responsibility of developing adequate analytical methods for detecting residues and furnishing them to the Government with a petition for approval of an additive. H.R. Rep. No. 2664, 86th Cong., 2d Sess. (1960).

The amendments proposed by the Department were not included in the color additive legislation. During the following 2 years, however, concern continued to be expressed about application of the anticancer clause in section 409. As a result, legislation similar to that earlier recommended by the Department of Health, Education, and Welfare was introduced in 1962. The House Committee on Interstate and Foreign Commerce ultimately included modifications of the anticancer clause in its report on the Drug Amendments of 1962, with the following explanation:

> The committee amended the anticancer clause of the food additives amendment and the color additive amendment of the Federal Food, Drug, and Cosmetic Act by making this clause inapplicable to chemicals such as veterinary drugs when used in feed for food-producing animals if the Secretary finds (1) that under the conditions of use and feeding specified in the proposed labeling and reasonably certain to be followed in practice, such additive will not adversely affect the animals for which such feed is intended, and (2) that no residue of the additive will be found (by methods of examination prescribed or approved by the Secretary by regulations) in any edible portion of the animal

after slaughter or in any food such as milk or eggs yielded by or derived from the living animal. H.R. Rep. No. 2464, 87th Cong., 2d Sess. (1962).

Although controversial, these amendments were agreed to by the full House of Representatives. The Senate accepted the House-passed modifications of the anticancer clauses in conference (H.R. Rep. No. 2526, 87th Cong., 2d Sess. (1962)).

Beginning in 1962, efforts were also made in Congress to consolidate the various provisions of the law applicable to animal drugs under the new drug, food additive, and antibiotic sections of the statute, with the objectives of clarifying the applicable requirements and expediting approvals of new animal drugs. No attempt was made to reopen the issue of the anticancer clause, however, and neither the committee reports nor the floor debates in the resulting legislation mentioned the anticancer clause which precluded approval of a new animal drug if:

> * * * such drug induces cancer when ingested by man or animal or, after tests which are appropriate for the evaluation of the safety of such drug, induces cancer in man or animal, except that the foregoing provisions of this subparagraph shall not apply with respect to such drug if the Secretary finds that, under the conditions of use specified in proposed labeling and reasonably certain to be followed in practice (i) such drug will not adversely affect the animals for which it is intended, and (ii) no residue of such drug will be found (by methods of examination prescribed or approved by the Secretary by regulations, which regulations shall not be subject to subsections (c), (d), and (h)), in any edible portion of such animals after slaughter or in any food yielded by or derived from the living animals * * *. (21 U.S.C. 360b(d)(1)(H).)

The legislation was enacted without controversy as the Animal Drug Amendments of 1968, and without evident congressional desire to alter the anticancer clauses, as modified in 1962 for animal drugs.

B. STATUTORY INTERPRETATION

The enactment in 1962 of the so-called DES proviso to the Delaney anticancer clause has been a source of continuing controversy. There has not been unanimity on the proper interpretation of Congress' action, and the legislative history of the proviso, summarized above, does not lay to rest all doubts.

Two interpretations of the proviso are, in theory, possible. The first interpretation, which in the Commissioner's judgment is the less probable, is that Congress intended to allow FDA to approve the use of a carcinogenic compound in food-producing animals only if it could be absolutely positive that no traces whatever—no matter how small—would remain in edible tissues.

This interpretation presents several difficulties, all stemming from the fact that any introduction of a compound (whether or not carcinogenic) is likely to leave minute residues in edible tissues that are below the level of detection of any known or likely to be developed method of analysis (assay). It is a fundamental fact of analytical science that for every assay developed to measure the concentration of a chemical compound in a medium (in this case, a residue in an edible tissue, there is some lowest concentration or level of such compound below which the assay will not yield an interpretable result. If, for example, an assay measures a particular compound in muscle tissue (an edible tissue), and the assay has been shown to have a lowest limit of measurement of one part per billion (1 ppb—one part compound in one billion parts tissue on a weight basis, such as 1 nanogram of compound per 1 gram of tissue), examination of muscle tissue using this assay will reveal that the compound is present only if its concentration in muscle tissue is 1 ppb or higher. If the compound is present in the tissue at levels below 1 ppb, use of the assay will yield no interpretable result. Thus, the assay cannot distinguish between muscle tissues containing the compound at levels below 1 ppb and muscle tissues from which the compound is absent in the absolute sense of the term.

Although different assays may have different lowest limits of measurement, all assays are subject to the same limitation. Thus, when a tissue is examined with an assay having a lowest limit of measurement of 1 ppb and no interpretable response is observed, the analyst can only conclude that the compound under analysis is not present at levels of 1 ppb and above. It can never be concluded that the compound is "not present" in the absolute sense. It is thus impossible to determine the conditions under which edible tissues derived from food-producing animals that have received a carcinogen will contain no residue if the phrase "no residue" is to be interpreted literally. Accordingly, this first possible interpretation of the DES proviso would not permit the approval of any animal drug known to be carcinogenic because the Commissioner could never find that no trace whatever would remain in the edible tissues of the animals to which the compound was administered.

This interpretation would thus render the DES proviso a "Catch-22." The proviso would permit the approval of carcinogenic drugs for animals if the Commissioner could be certain that no residues whatever would remain, but since he would only conclude that some trace might well remain, no such drug could ever be approved. This seems, at the very least, an improbable interpretation of an amendment Congress enacted precisely because it wanted to relieve animal drugs from the rigid strictures of the anticancer clauses.

Furthermore, this interpretation is difficult to reconcile with the language of the DES exception, which specifies that "no residue" may be "found (by methods of examination prescribed or approved by the Secretary * * *) in any edible portion of such animals * * *." This language conspicuously avoids such words as "occur" or "remain," and instead emphasizes detectability. Moreover, the same proviso refers to "conditions of use * * * reasonably certain to be followed in practice," suggesting a congressional recognition that the occurrence of some residues, i.e., residues resulting from unforeseeable misuse, might not require disapproval of a compound even if they were detected.

A second, and in the Commissioner's view more plausible, interpretation of the DES proviso accepts the words of the amendment and focuses on the language previously quoted: "no residue of such drug will be found (by methods of examination prescribed or approved by the Secretary by regulations * * *." Under this interpretation, an animal drug that is carcinogenic may be approved for use in animals if examination of edible tissues by an assay approved by FDA reveals no residues.

This in essence is the interpretation that FDA has followed since the passage of the DES proviso: The agency has approved carcinogenic compounds for use in animal feed or as animal drugs on the basis of assays capable of measuring prescribed levels of residues. However, the agency has not previously attempted to define and explain the criteria it employs in evaluating assays submitted in support of approval of animal drugs, feed additives, and color additives. That is the purpose of this document.

The Commissioner believes that the criteria to be applied in evaluating assays for residues of carcinogenic compounds in the edible tissue of food animals must further the congressional objective of minimizing public exposure to carcinogenic compounds, without nullifying the decision reflected in the DES proviso, which the first interpretation of the proviso would do. As explained more fully below, the criteria set forth in these regulations for the evaluation of assays for carcinogenic residues are minimum requirements. They are designed to identify assays that are (1) reliable and practical for use by a regulatory agency and (2) capable of measuring residues at levels that have been determined, on the basis of animal toxicity tests, to present no significant increase in human risk of cancer. An assay that does not meet both criteria cannot be approved. The Commissioner recognizes that for some compounds currently in use no reliable and practical assay capable of sufficiently low limits of measurement now exists, and that approval of their continued use must be reexamined.

The Commissioner further believes that the policy embodied in the anticancer clauses requires application of a third criterion to the evaluation of assays: The agency therefore will insist that of the available assays, the one approved for controlling carcinogenic residues must be the one having the lowest limit of reliable measurement and capable of satisfying the other two criteria. This means that, as new practical assays capable of reliably measuring lower levels of residues become available, approved compounds will be controlled with such assays and petitioners will be required to make any modifications in the conditions of use of a compound necessary to prevent residues from occurring.

The Commissioner recognizes that this third criterion may lead to the withdrawal of approval of some compounds because they cannot be used without detection by newer assays. (This prospect is in part theoretical, however, because the other minimum criteria defined in this regulation demand a low limit of measurement for assays that for many compounds is at or below the lower limits of present technology.) Any other posture, however, would place FDA in the position of approving the use of carcinogenic compounds that could be measured by new, practical assays capable of reliably measuring lower levels of residues.

It is, of course, also true that the criteria outlined in these regulations will sometimes permit the approval, for use in animal feed or as animal drugs, of carcinogenic compounds that are likely to leave miniscule residues below the lowest level of reliable measurement of any assay that meets the other criteria herein set forth. This, however, is the result of congressional enactment of the DES proviso. Moreover, this result makes sense in practical terms, for a regulatory agency cannot effectively control residues—of any compound—that are so small that they escape measurement by every current assay, simply on the assumption that such residues must be occurring.

In sum, the interpretation adopted in these regulations is reconcilable with both the purpose and language of the DES proviso, and will further the congressional objective of minimizing public exposure to residues of carcinogenic compounds.

C. OVERVIEW OF THE REGULATION

The proviso to the anticancer clauses allows the approval of the use of carcinogens in food-producing animals if, under conditions of use "reasonably certain to be followed in practice," no residue is found by an (assay) prescribed or approved by the Secretary. To assure protection of the public in a manner consistent with the anticancer provisions of the act, the Commissioner must establish criteria for approval of assays to include, among other things, a required lowest limit of measurement.

Accordingly, these regulations establish criteria for accepting assays used to measure carcinogenic residues in edible tissues of food-producing animals which have been administered carcinogens. Such criteria cover assay attributes such as dependability, practicability, specificity, accuracy, and precision. Additionally, the regulations establish a specific criterion for the lowest limit of reliable measurement which an assay must meet, as a minimum, before it can be approved by the agency for the control of carcinogenic residues. This criterion for the required lowest limit of measurement of an assay derives from toxicological data obtained for carcinogenic residues and from an operational definition of the no-residue objective standard of the act. Only if an assay meeting the above criteria is available does the Commissioner have a mechanism to discriminate between tissues containing a residue and tissues containing no residue. Without such a monitoring mechanism, the commissioner has no way to determine if a carcinogenic drug or additive administered to a food-producing animal is or even can be used in compliance with the act.

In these regulations the Commissioner has estabilshed a rigorous premarket testing process for sponsored compounds intended for use in food-producing animals. The process treats all compounds initially as potential carcinogens and embodies conservative assumptions at each stage of the inquiry to determine the minimally acceptable lowest limit of reliable measurement for a regulatory assay. Because this minimally acceptable limit is determined by toxicity data, the Commissioner may conclude that an assay satisfying the requirements of the regulations is capable of demonstrating the absence of carcinogenic residues in food. By thus particularizing the statutory requirements, the Commissioner has established the basis for rejecting sponsored compounds which are claimed to satisfy the no-residue standard by other mechanisms.

1. *Fundamental questions.* For every drug or additive proposed for use in food-producing animals (hereinafter the sponsored compound), the Commissioner is required by the act to determine whether such sponsored compound can be used in ways which are safe for the animals to which the compound will be administered (target animals) and whether food (meat, milk, and eggs) derived from such animals (hereinafter edible tissues) will be safe for human consumption. The sponsor of such compound (hereinafter the petitioner) is therefore required to furnish the Commissioner the scientific and technological information necessary for such a determination; the Commissioner in turn is required by the act to determine on the basis of all available data whether, in actual practice, the sponsored compound can be used in compliance with the law.

Although a major obligation of a petitioner proposing the use in food-producing animals of a compound that is a carcinogen is the development of a practical and reliable assay capable of discriminating tissues containing residues from tissues free of such residues, as defined operationally, such as an assay cannot be developed in the absence of certain scientific and technological information whose nature is not strictly analytic.

Specifically, for every sponsored compound, several questions must be answered before assay development can be undertaken or compound approval considered:

(a) What is the chemical nature of the sponsored compound and how is it to be used?

(b) On the basis of preliminary toxicological and biochemical information, can it be concluded that the compound has the potential to contaminate human food (edible tissues) with residues of carcinogenic concern?

(c) If so, what is the chemical nature of the residues of the compound, in what tissues are they found, at what levels, and for what length of time?

(d) Is the sponsored compound or any of the residues it produces in edible tissue carcinogenic in experimental animals?

(e) If so, what level of residues can be operationally defined as satisfying the no residue requirement of the act?

(f) Can a reliable and practical assay be developed to measure the edible tissue residues at a level at least as low as that which operationally satisfies the no-residue requirement of the act?

(g) At what time after cessation of compound exposure do the edible tissues of exposed food-producing animals satisfy the no-residue requirement of the act, i.e., what is the necessary withdrawal time?

2. *Data collection process.* To provide answers to the preceding questions, a petitioner must gather pertinent scientific information, the nature of which is particularized below. These regulations establish the procedure for gathering and evaluating the requisite scientific information. The process is stepwise and evolutionary because the need, as well as ability, to proceed to the next step of data collection depends upon the results obtained at each preceding step. If the evaluation of the data collected at each step indicates that questions regarding residues of carcinogenic concern remain, the process of data collection must continue. If at some point in the process of data collection it can be decided that the sponsored compound presents no human risk of carcinogenesis, the sponsored compound shall be evaluated under the general food safety provisions of the act. In such a case, the compound may be assigned a safe tolerance level in human food if the petitioner provides the data necessary to establish that the compound can be used safely.

These regulations deal with carcinogenesis, which is a dominant concern in appraising the safety of any sponsored compound intended for use in food-producing animals. Nevertheless, each compound must also be evaluated for other potential adverse effects. Thus, for example, if the available information raises issues concerning the health of progeny, multigeneration studies of the sponsored compound and/or its residues shall be codesigned and conducted as a part of the process of data collection and evaluation.

If the Commissioner makes a threshold determination, based on (1) preliminary biochemical, chemical, toxicological and physiological data, and (2) proposed patterns of use, that a sponsored compound has the potential to contaminate food from food-producing animals with residues whose consumption would pose a human risk of carcinogenesis, the petitioner will be required to undertake the following six-step procedure for data collection and evaluation.

(a) A metabolic study in the target animals designed to identify edible tissue residues of carcinogenic concern.

(b) A metabolic study of the sponsored compound in experimental animals designed to aid in assessing the carcinogenicity of residues that can not practicably be tested individually (so-called "intractable residue").

(c) Chronic toxicity testing to assess the carcinogenic potential of residues of the sponsored compound and to furnish data suitable for statistical treatment to permit the no-residue requirement of the act to be defined and implemented.

(d) A detailed metabolic study of the sponsored compound in target animals designed to identify a residue and tissue that can serve as indicators ("marker residue" and "target tissue") to determine whether the no-residue requirement of the act is satisfied.

(e) Development of a regulatory assay to measure the marker residue in the target tissue at and above the level established in step (d).

(f) Establishment of the premarketing withdrawal period required for the safe use of the sponsored compound.

Because the partial provisos to the anticancer clauses of the act, sections 409(c)(3)(A), 512(d)(1)(H), and 706(b)(5)(B), although varying slightly in their language, have a similar intent, the Commissioner has concluded that the criteria for their implementation should also be identical. To avoid needless repetition, however, where appropriate the Commissioner has used the language of section 512 of the act in discussing specific generic issues because the primary impact of these regulations will be on new animal drugs regulated under that section. The criteria set forth in these regulations shall, however, apply to all chemicals intended for use in food-producing animals, and the appropriate regulations will be amended to adopt these criteria by reference.

Since issuing the proposal under § 135.38 (21 CFR 135.38), FDA has recodified all regulations applicable to animal products in Subchapter E of Title 21 of the Code of Federal Regulations to provide space for the orderly development of future regulations and to provide the public and other affected parties with regulations that are easy to find, read, and understand. For these reasons, the final order has subdivided the proposal into 10 individual regulations and established a new subpart in Part 500, Subpart E—Criteria and Procedures for Evaluating Assays for Carcinogenic Residues in Edible Products of Animals.

II. Threshold Assessment

In the 1973 notice of proposed rulemaking, the Commissioner proposed that carcinogenicity testing not be required for every sponsored compound. Rather, he concluded that the necessity for such testing will be dictated by an evaluation of the existing evidence from metabolic studies, standard toxicity testing, structural relationships of the sponsored compound and/or its metabolites to known carcinogens, modes of physiological actions and interactions, and the intended method of use of the sponsored compound.

Comments of two types were received on this feature of the proposal. The first suggested that extensive studies should be conducted for every sponsored compound to determine whether it is a carcinogen. One comment insisted that extensive carcinogenesis testing for every sponsored compound is the only accurate indicator of carcinogenic potential. Several contended that the criteria proposed for use in the threshold determination were too vague, and objected to the lack of explanation of how such criteria could be applied in practice.

Many other comments agreed with the Commissioner's proposal that extensive carcinogenicity testing should not be required for every sponsored compound. These comments recommended that the Commissioner review all available data pertaining to a sponsored compound before he concludes that the stepwise testing procedure set forth in the proposal and adopted in this regulation should be invoked.

When a petitioner initiates the process of gaining approval for use of a compound, information is provided to the agency on matters such as compound efficacy and its proposed patterns of use. Often a petitioner will also provide preliminary physiological, metabolic, or toxicological data derived from its own studies or from the scientific literature. At this juncture, the Commissioner believes it necessary that a threshold assessment be made, based on the available data, on the need to proceed to the first of the six steps of data collection required by these regulations. Because entry into the six steps of data collection requires that a petitioner undertake a series of very complex and costly experimental studies, imposing demands on the limited national resources available for determining the safety of chemicals entering the environment, the Commissioner concludes that it is not reasonable to demand such studies on a sponsored compound if the preliminary data available justified the judgment that public health can be protected without so proceeding.

Criteria for this threshold assessment cannot be elaborated in detail. The Commissioner must examine the available preliminary data, which may vary considerably in quality and content from one compound to the next, on a case-by-case basis and determine whether a sponsored compound has the potential to contaminate edible tissues with residues of carcinogenic concern. However, certain general characteristics of the compound shall always be considered in making the threshold assessment:

(1) Is the compound a known carcinogen or is it related, in a chemical or biological sense, to other known carcinogens?

(2) Is there an indication in preliminary toxicity studies that the sponsored compound may be carcinogenic?

(3) Does preliminary information on the fate of the compound in target animals indicate that, in combination with information on the proposed pattern of use, there is a high or low probability that residues can occur in edible tissues when such tissues become available as food?

In making a threshold assessment, the Commissioner may or may not have answers to these questions and, in some instances, may not need answers to all of them to make a decision. It will sometimes be obvious that the first step of the six-step process will have to be undertaken. In other cases, it will be equally clear that no such inquiry need be begun, and the compound can be evaluated under the general food safety provisions of the act. Finally, in some cases, available information will be so incomplete or ambiguous that a decision will be made to move to the first step to assure protection of public health. As will be shown later, it is possible that information developed in later steps may support or require revision of the threshold assessment that a compound had the potential to contaminate tissues with residues of carcinogenic concern, in which case the remaining steps of these regulations will not be required and evaluation will proceed under the general food safety sections of the act.

The following examples illustrate how a threshold assessment can be made:

Case I.—A drug is proposed for use in day-old chickens. Preliminary information indicates that:

(a) Neither chemical structure nor preliminary (short-term) toxicity testing raise a suspicion that the drug is a carcinogen.

(b) The drug is proposed for therapeutic use only in a single administration to day-old birds.

(c) The disease to be treated occurs infrequently.

(d) Preliminary metabolic data indicate accumulation of residues in kidney and no detectable residues in muscle.

(e) Residues deplete rapidly and none are detected many weeks before the chickens reach marketing weight.

If presented with the foregoing information, the Commissioner would see no justification for demanding that the petitioner proceed to the first step of these regulations which governs compounds having the potential to contaminate edible tissues with residues of carcinogenic concern. However, if the preliminary metabolic study in the example had been conducted with an assay having a lowest limit of reliable measurement of residues substantially higher than current technology can attain, the Commissioner would conclude that the available data were insufficient to justify a favorable threshold assessment about the sponsored compound, and the petitioner would be required to proceed to the first step of these regulations. It is precisely because of such contingencies that the Commissioner concludes that no more specific criteria for threshold assessment should be established by regulation.

Case II.—A drug having growth promoting properties is proposed for use in cattle. The preliminary information indicates that:

(a) The observed physiological activity of the drug in cattle indicates that it is

in a class of other known carcinogens whose carcinogenic properties appear to be related to this particular physiological activity (i.e., the drug is a suspect carcinogen).

(b) The drug is used during a large fraction of the lifetime of the animal.

(c) The drug is likely to be widely used in animal husbandry.

(d) Preliminary metabolic data show that residues of the drug accumulate in muscle tissue (meat) and deplete very slowly. On the basis of such information, it is obvious that the Commissioner would have to require the petitioner to proceed to the first step of the required six-step process.

III. Metabolic Study in Target Animals To Identify Residues of Concern

A. Need to identify residues in edible tissue

Before any decision can be made concerning conditions of safe use of a sponsored compound, it is necessary to obtain information on the residues that occur in edible tissues when the compound is administered to the animals for which it is intended (target animals). Without such information, rational decisions about the human safety of edible tissues derived from treated animals are not possible.

A compound administered to an animal can be acted upon by the enzymatic systems or physiological fluids of the animal and new compounds (metabolites and degradation products of the sponsored compound) are produced in the process. Therefore, the sponsored compound is not the only tissue residue of concern. And sections 512(b)(7) and 512(d)(2) of the act explicitly require the Commissioner to consider the safety of any substance formed in or on food by a sponsored compound before approving its use.

Numerous comments were received on the proposal's requirement for metabolic studies. Several comments stated that there should be no attention paid to metabolites. Others contended that metabolism studies should not be routinely required, on the ground that the pathway of excretion is of no toxicological importance if all of the administered compound has been eliminated from the tissues of the target animal. Most comments recommended that a metabolism study should only be required to determine the major metabolites in the edible tissue of target animals, suggesting that the public health would not be served if petitioners are required to pursue endless structural elucidations and quantitations of all metabolites even though some of them might constitute minor fractions of the residue of the sponsored compound. Comments also contended that it may not be experimentally possible to administer to animals sufficient quantities of a compound to obtain amounts of residues sufficient for structural identification. Several comments asserted the studies should be limited to identification of residues in the edible tissues of target animals and that generally it would be unnecessary to have such information on metabolites in inedible tissues. Further, some comments stated that radiotracer studies can be employed to determine the time by which the sponsored compound and its metabolic products are eliminated ("out time"). However, other comments suggested that all metabolites should be identified and tested for toxicity.

The Commissioner reiterates that the objective of requiring metabolic studies is to assure collection of sufficient scientific information on residues to permit a food safety evaluation which in turn can be used to establish parameters for regulatory assays. Therefore, he has concluded that the following metabolic studies are necessary to permit a determination of whether the proposed use of a sponsored compound is safe.

B. Conduct of metabolic study

1. *Test animals.* The metabolic fate of an administered compound in an animal may be unique for each livestock production class. Therefore, the Commissioner concludes that a metabolic study in the animals for which a sponsored compound is intended (target animals) is necessary. If the petitioner can demonstrate that the data from the metabolic study obtained for one production class are applicable to a second, the Commissioner may modify the extent of investigation required for the latter.

2. *Required technology.* Because the metabolic fate of a compound administered to food-producing animals plays a pivotal role in decisions regarding the need for an extent of carcinogenesis testing required to assure public health and safety, it is mandatory that such fate be adequately determined, i.e., it must be demonstrated that residues of potential carcinogenic significance have been detected at levels obtainable by the best analytical technology available. Therefore, the Commissioner concludes that the required metabolic studies shall be conducted with the best analytical methods technology can provide.

As will be seen in part VI of this preamble, it is necessary to select one residue that can serve as a practical indicator to assure that the no-residue requirement of the act is met. Such a residue can only be selected by reference to a metabolic study in which residues are detected and measured at levels dictated by the outcome of actual carcinogenicity testing. Because these levels cannot be known at the outset of this phase of the metabolic study in target animals and because the "best available technology" may not be adequate to measure the levels dictated by the outcome of carcinogenicity testing, it may be necessary to develop improved technology and to repeat the metabolic study in target animals, after carcinogenicity testing has been completed. Another requirement of the second metabolic study will be the development of enough data to construct tissue concentration-time profiles for some residues.

3. *Analytical techniques.* For the foreseeable future, the general technique of choice for metabolic studies will be the use of radiotracers. The regulations, therefore, recommend that the required metabolic studies be conducted with radiolabeled compounds of the highest specific activity that is available and is consistent with principles that assure scientific quality. These principles concern the types, the chemical nature, the chemical and metabolic stability, and the suitability of radiolabels for metabolic studies having specific objectives. They have been developed from past metabolic studies with radiotracers and should be followed to assure the scientific quality of the required metabolic studies.

The task of experimental residue detection can often be made easier by available information on the metabolism of related compounds. It is recommended that metabolically feasible pathways applicable to the sponsored compound be proposed based on relevant literature references about compounds of similar structure. This information can usually simplify the choice of radiolabel positions which will assure that all residues containing structural moieties of potential toxicological concern can be detected. However, such projections of likely metabolism can never be a substitute for experimental observation of the metabolic fate of the sponsored compound.

Although the use of radiotracers is the preferred experimental procedure, some compounds possess inherent physicochemical characteristics (e.g., strong fluorescence associated with the structural moiety of potential toxicological significance) that will allow the necessary detection of residues. In such cases, the use of radiolabels may not be required.

4. *Dose regimen.* The dosing regimen for the metabolic study in the target animals shall be consistent with the maximum proposed use level and duration of exposure to the sponsored compound. For compounds administered continuously over long periods of time, administration for the metabolic study need continue only until equilibration or saturation of edible tissues has been demonstrated.

The metabolic fate of a compound administered to target animals is likely to depend on the conditions (level, method, and duration) of use. Because the purpose of the required metabolic studies is to characterize and quantitate residues under conditions of proposed use, these conditions shall be followed in the metabolic studies. However, it is possible that under such conditions certain residues are produced in amounts that do not allow extensive chemical characterization. If the structure of any such residues must be determined, and residues can be produced in sufficient amounts by administering to target animals larger doses of the sponsored compound, the petitioner will be allowed to follow this procedure. In some instances, chemical synthesis of residues may be more feasible, especially if they are needed for chronic toxicity testing.

5. *Required data.* Since the relative persistence of residues in edible tissues is

one consideration in selecting specific residues for toxicity testing, the regulations require that the total number and the relative quantities of residues shall be determined immediately following cessation of treatment, as well as some later time. The Commissioner has concluded that the identification process shall ordinarily continue until the total residue burden in the edible tissues of the target animals has depleted through at least three half-lives. After such time, it is unlikely that new residues previously undetected will appear to alter the residue picture.

The need for and extent of chemical characterization of residues depend on a number of factors. Ordinarily, compounds that constitute a significant fraction of the total residue require sufficient physical and chemical characterization to ascertain whether or not a structural change has taken place which could increase the carcinogenic potency of the residue over that expected of the sponsored compound. In some instances, it may be impossible to judge whether the residue has carcinogenic potential, but significant structural alteration alone may be enough to signal the need for further characterization. Since such structural changes are not uncommon during metabolism and since it is the tissue residues to which human beings will be potentially exposed, such characterization will normally be required. When the agency determines a component of the residue requires chronic toxicity testing (because of tissue concentration and persistence and/or expectation of increased carcinogenic potential), chemical characterization will ordinarily have to be complete and an effort to obtain sufficient quantities of the residue(s) for toxicity testing will be necessary. (See, however, paragraph III.C., below in this preamble.)

In some instances, a petitioner may be required to pursue the complete characterization of certain relatively minor metabolites if partial physiochemical characterization indicates that a structural change during metabolism in the target animal has introduced molecular moieties of carcinogenic potential greater than that expected of the sponsored compound, e.g., nitrosation of an amine of unknown carcinogenic potential to product nitrosamines of known carcinogenic potential.

Because uncharacterized tissue residues pose a risk to public health, the regulation requires that the procedures for separation, purification, and characterization be consistent with the best available scientific and technological capabilities. Ordinarily, the agency will require attempts at characterization to include use of a variety of procedures based on the various forms of chromatography, spectroscopy, and spectrometry.

6. *Format for data submission.* The Commissioner has concluded that the format for presenting results of metabolic studies should be standardized to minimize possibility for misinterpretation of data. Because these studies will provide the basis for major public health decisions, the Commissioner considers it essential that they be carried out and reported in a manner consistent with the best available criteria. The two professional societies listed in the regulations (American Chemical Society and American Society of Biological Chemists) follow policies for acceptance of manuscripts that embody the best available criteria for collecting, interpreting, and reporting scientific data of the type required by this regulation.

C. COMPARATIVE METABOLISM STUDY TO AID IN ASSESSING CARCINOGENICITY OF INTRACTABLE RESIDUES

1. *Sponsored compound always tested: Rationale and procedure.* When it is determined that a sponsored compound has the potential to contaminate edible tissues with residues whose consumption may pose a human risk of carcinogenesis, the sponsored compound itself shall always be tested for carcinogenesis. Residues are selected for testing according to those criteria already discussed in paragraph III.B., but there are overriding reasons for testing the sponsored compound, even if it is not detected as a residue. Metabolic transformation or nonenzymatic degradation of a sponsored compound can lead to a number of tissue residues which cannot be obtained (either by isolation or synthesis) in sufficient amounts for carcinogenicity testing (such residues are herein and in the regulation referred to as "intractable residues"). Testing the sponsored compound itself therefore provides one experimental means for acquiring data on the carcinogenic potential of such residues.

Although the dominant criterion for selecting test animal species or strains for chronic toxicity testing will be the degree to which a species or strain models man, the application of a secondary criterion for selection can provide a means for addressing the problem of intractable residues. Specifically, selection of test animals can also be based on comparative metabolism data (target animal and test animal) which can be used to determine the extent to which particular species or strains, by virtue of the way they metabolically convert the sponsored compound, will be exposed during testing to the same complement of residues expected in tissues derived from target animals.

For example, if a metabolite detected as a residue in edible tissues of the target animal is determined to be toxicologically important, the petitioner will be asked to pursue isolation or synthesis of the compound for toxicity testing purposes. If all attempts at this fail, then the comparative metabolism approach is available if a potential test animal species is shown to produce the same metabolite when it is administered the sponsored compound. In this way, there is some degree of assurance that the toxicity test of the sponsored compound also provides some estimate of the toxicity of the intractable metabolite. Because human food could be contaminated with the intractable metabolite, such a test provides a practical approach to a complex and important issue.

This construct has been included in the final regulations in response to comments that either suggested that all metabolites ought to be ignored (which the Commissioner concludes is neither legally nor scientifically acceptable) or that all metabolites must be isolated and independently tested (which is not technologically possible).

2. *Selection of residues for chronic toxicity testing.* On the basis of all of the studies described above, the Commissioner will select those residues, in addition to the sponsored compound, that require chronic toxicity testing.

IV. CHRONIC TOXICITY TESTING

The sponsored compound and any residues selected for testing shall be subjected to oral, lifetime, dose-response studies in two of the test animal species/strains selected in accordance with the criteria described in the foregoing paragraphs. The purpose of these studies is to determine if the compounds under test are carcinogenic and, if so, to establish the lowest limit of reliable measurement that must be achieved by any regulatory assay for monitoring residues resulting from use of the sponsored compound.

Several comments on this feature of the proposal dealt with the testing of chemical compounds for carcinogenic potential, and addressed two major issues: (i) The design of chronic studies, and (ii) the relevance of animal testing in evaluating human safety.

The Commissioner appreciates the inherent complexity of these issues. He further recognizes that they are common to many areas of food safety, as well as environmental safety, and must be dealt with in an integrated manner in forthcoming regulations on general food safety. However, he believes some discussion of these issues must be included in this preamble as they relate to the context of this regulation.

A. DESIGN OF CARCINOGENICITY STUDIES

Comments on the proposal expressed a variety of contrasting opinions regarding the design features of carcinogenicity studies with experimental animals. The comments specifically addressed: (i) selection of appropriate test animals; (ii) conditions, levels, and duration of exposure; and (iii) statistical design as it relates to number of animals in bioassay, distribution of animals to the various levels of exposure, and adequacy of controls.

The Commissioner recognizes that the impact of these design features on the meaning of animal carcinogenesis data is an important and controversial matter that is currently the subject of intense scientific investigation. The major effort at FDA's National Center for Toxicological Research is specifically directed towards development of relevant protocols and experimental designs for carcinogenicity testing. Until these efforts are concluded and the results incorporated into regulations, the Commissioner recommends that guidance be found in the

report of the Food and Drug Advisory Committee on Protocols for Safety Evaluation: Panel on Carcinogenesis, Report on Cancer Testing in the Safety Evaluation of Food Additives and Pesticides ("Toxicology and Applied Pharmacology," 20:419–438, 1971). This report reviews and analyzes all facets of experimental design that have been developed and scrutinized by competent scientists prior to 1971. To facilitate incorporation of later developments in testing standards as they have and will evolve, the regulations suggest that petitioners submit developed protocols to the Commissioner for review and updating prior to initiating studies.

B. RELEVANCE OF ANIMAL TESTING IN EVALUATING POTENTIAL FOR HUMAN CARCINOGENESIS

Several comments on this aspect of the proposed regulation dealt with the merits of animal testing as an experimental tool. Some comments pointed out that even animal testing done under the best experimental protocols can never prove conclusively that a compound is not carcinogenic, and that under such circumstances, some weak carcinogens are likely to escape identification. Other comments expressed the contrasting view that adequate protocols can be devised. Still others questioned the propriety of drawing conclusions about human carcinogenesis from data collected with experimental animals.

The act requires that in assessing the safety of animal drugs, the carcinogenic potential of residues shall be evaluated. Ordinarily, such evaluation must be based on appropriate testing. Given the gravity of the decisions that depend on the results of such evaluations, the best relevant scientific information must be developed and assembled. As a source of information, direct carcinogenesis testing of chemical compounds in man is and must remain beyond the ethical bounds placed by society on human experimentation. In the absence of this source of information, which incidentally would be most relevant, alternate sources are human epidemiology studies and animal experimentation. Human epidemiology may provide post facto information about the carcinogenic effects of chemical compounds on man. However, while potentially useful in assessing the significance of new exposures or the risk posed by related compounds, such experience cannot be a central basis for food safety evaluations for several reasons, including the same ethical objections that make direct experimentation in man unacceptable.

The Commissioner therefore concludes that the agency must continue to rely on animal testing for the evaluation of the safety for humans of chemical compounds proposed for use in food-producing animals. Moreover, the act does not distinguish between compounds demonstrated to be carcinogenic in test animals and human carcinogens. Instead, it assumes without proof that an animal carcinogen may be carcinogenic in human beings. In this context, the issue of relevance to man of data from tests in animals must be refocused. The regulatory objective must be to avoid falsely negative determinations of the carcinogenic potential of compounds under test in experimental animals that are appropriate models for man. In this setting, the only tenable regulatory posture for the agency is to select bioassay protocols which utilize test-animal species/strains that have the greatest possible susceptibility to the test compound and are also appropriate models for man. Available toxicologic and metabolic information shall provide a basis for such selection.

C. INTERPRETATION OF TEST DATA—IS THE COMPOUND A CARCINOGEN?

The objective of collecting and interpreting test data is to decide whether or not the compound under test (the sponsored compound and any selected metabolites) is a carcinogen. Within certain limits of confidence, statistical treatment of chemical carcinogenesis data can provide objective criteria for such determinations. To the question "Is the tested compound a test-animal carcinogen?" statistics can provide one of two types of answers:

(i) With "x" percent confidence (i.e., in "x" cases out of 100), "y" dose of the test compound will increase the carcinogenesis risk of test-animals over controls by no more than "s" and no less than "t"; or

(ii) With "x" percent confidence, "y" dose of the test compound will increase carcinogenesis risk of test animals over controls by no more than "s."

Answers of the first type are possible only when the observed incidence of carcinogenesis in the test animals is significantly greater than that in the controls. When the observed incidence is the same for test and control animals, only answers of the second type are possible.

A statistically significant increase in the incidence of carcinogenesis in test animals (i.e., an answer of the first type) is sufficient evidence to classify the test compound as a test-animal carcinogen. Because the act does not distinguish between human and animal carcinogens, for the purpose of these regulations, classification of a test compound as a test-animal carcinogen brings into play the requirements of the anticancer clauses. Revisions of such classification on the basis of phyllogenetic considerations can have no bearing on the applicable legal requirements.

If the animal test data will permit only answers of the second type, the decision whether to classify the test compound as a test-animal carcinogen is more difficult. A negative test finding, as pointed out in some comments, can mean either that the test compound is not a test-animal carcinogen at the tested dose, or that the bioassay protocol lacks a sufficient number of animals, or animal susceptibility, or both, to discern an increase in the risk of carcinogenesis in the test animals. In such cases, a decision must be made whether to classify a tested compound as a noncarcinogen or to require further experimentation appropriate for resolving questions of safety.

V. OPERATIONAL DEFINITION OF THE NO-RESIDUE REQUIREMENT

A. ALTERNATE OPERATIONAL DEFINITIONS

If its has been determined that a sponsored compound, when administered to food-producing animals, has the potential to contaminate edible tissue with residues whose consumption may pose a risk of human carcinogenesis, the agency cannot approve the sponsored compound unless it can be demonstrated that conditions of use can be established that ensure the no-residue requirement of the act can be met. To establish such conditions of use and to provide a means for ascertaining whether these conditions are met in actual practice, some operational definition of the term, "no residue," is necessary. Indeed, the act contemplates that the Commissioner will provide such an operational definition, for he must have some criteria for prescribing or approving methods of examination for measuring residues.

The Commissioner has considered three alternate approaches to an operational definition of the phrase. Under one approach the term, "no residue," might be operationally defined as satisfied when the levels of residues fall below those that can be measured by available analytical methodology (alternative 1). A second approach would be to establish some low finite level (e.g., one part per billion) as a "practical zero" and to require assays that can reliably measure this "zero," insisting on the development of new assays if available assays were not adequate (alternative 2). Finally, "no residue" might be operationally defined on the basis of quantitative carcinogenicity testing of residues and the extrapolation of test data using one of a number of available procedures to arrive at levels that are safe in the total diet of test animals and that would, if they occurred, be considered safe in the total diet of man. Under this approach, the Commissioner would require assays that can reliably measure that safe level in edible tissues (alternative 3). For the reasons discussed in section V.B. of this preamble, the Commissioner has concluded that alternative 3 should be adopted. The results of the carcinogenicity testing of the sponsored compound and any selected residues shall be treated by the statistical procedures described in this part V and prescribed in § 500.87 (21 CFR 500.87).

B. CHOICE OF AN OPERATIONAL DEFINITION

1. *Alternative one.* A number of assays might be developed to measure the concentration of a chemical compound (i.e., residue) in an edible tissue, but for each there would be some level below which the compound under analysis could not be measured. (See section I.B. of this preamble). Generally, different assays for the same chemical compound will have different, and sometimes vastly difficult, lowest limits of measurement. The "no residue" requirement of the act could be translated into an operational definition that is based solely on available analytical methodology and specifically on the lowest limit of measurement of an avail-

able assay. Thus, the degree of public risk associated with the use of a sponsored compound would become a function solely of the capability of available analytical technology.

The Commissioner concludes that this approach is unsound because it ignores all quantitative aspects of carcinogenicity testing. The carcinogenic potency of different chemicals varies widely; failure to consider this fact in developing criteria for the evaluation of sponsored compounds would be scientifically unsound. It could produce situations in which residues of extremely potent carcinogens were not measured in edible tissues at levels as low as the measurable levels of residues of relatively weak carcinogens, if the assay available to measure the former happened to have a lowest limit of measurement that was higher than that of the assay available to measure the latter. Accordingly, failure to consider quantitative carcinogenicity data in establishing the criterion of lowest limit of measurement that an assay must meet would be tantamount to ignoring public health protection in evaluating the use of sponsored compounds.

2. *Alternative two.* A second approach the Commissioner has considered would be to establish "practical zero" for the residues of all carcinogens. This approach would have one advantage over alternative one; it would provide a well-defined criterion for the lowest limit of measurement that any petitioner's assay would have to satisfy. This approach would not, however, take into account differences in carcinogenic potency among various carcinogens and is therefore unacceptable for the same reason as alternative one.

Under alternative two the criterion for lowest limit of measurement would reflect consideration of what lowest level of measurement is "practical," given the state of the art of analytical chemistry or biochemistry. In addition to failing to link the no-residue standard to any consideration of carcinogenic potency, this approach fails on the ground of practicality. The science and technology of analytical chemistry and biochemistry are continuously changing, and a lowest limit of measurement which might be considered reasonable at one time would have to be discarded as unreasonable at some later time. Whenever a new and lower criterion for the limit of measurement were established, it would be incumbent upon the Commissioner to then require that use of all compounds approved under the prior criterion be suspended until methods were developed to measure the residues at this lower level. Such a situation, in the Commissioner's judgment, would be both unreasonable and unmanageable.

3. *Alternative three.* A third approach to defining operationally the no-residue requirement is to establish a required lowest limit of measurement for each sponsored compound on the basis of data derived from carcinogenicity testing of the compound and selected metabolites. Under this approach carcinogenic potency is given specific consideration because actual chronic toxicity test data are used to determine the level of residues in edible tissue that an assay must be capable of reliably measuring. Thus, it permits a rational, uniform procedure for establishing the required lowest limit of measurement for assays and avoids the major deficiencies inherent in alternatives one and two.

Should new information relating to the carcinogenic potency of residues of a sponsored compound later appear, this approach provides a practical basis for determining whether a new assay is required to establish compliance with the no-residue requirement. But only under such circumstances will it be necessary for the Commissioner to insist that the petitioner develop a new assay; thus, this approach contributes to regulatory stability and predictability. If an assay becomes available with a lowest limit of measurement that is lower than the level required by the analysis of quantitative carcinogenicity data, the Commissioner will adopt that method if it also meets the other rigorous criteria described in part VIII of this preamble and § 500.90 (21 CFR 500.90). However, for compounds that have been approved for use on the basis of an assay that satisfies the requirements of the regulation, the development of such a method will not be required. Thus, following this approach, the Commissioner can provide the maximum public health protection based on both quantitative carcinogenesis data and improved analytical technology. For these reasons, the Commissioner concludes that alternative three is the most rational approach to developing an operational definition of "no residue."

By adopting this approach to implementing the "no residue" standard, the Commissioner has assumed that: (i) The carcinogenic potency of chemical compounds can be quantified, and (ii) a dietary level of a carcinogen can be identified at which no significant human risk of carcinogenesis would derive from consumption of food containing residues below this level.

The carcinogenic potency of compounds can be determined by testing in experimental animals, although such determinations are subject to known limitations inherent in every measuring device or system. The second assumption, that potential residue levels representing no significant human risk of carcinogenesis can be assigned, is controversial, but it must be fully confronted and resolved if the public is to be protected from the potential and real dangers that inhere in the interpretations of the no-residue standard of the act outlined as alternatives one and two.

C. ANALYSIS OF ANIMAL CARCINOGENESIS DATA TO DEFINE OPERATIONALLY THE NO-RESIDUE STANDARD OF THE ACT

1. *Introduction.* The modified extrapolation procedure of Mantel and Bryan proposed for use in defining the no-residue standard for a sponsored compound is a statistical technique that allows estimation of the level, or dose, or a carcinogen that would lead to cancer incidence rates in test animals well below those rates that can be detected in practical experimentation. In normal experiments in which test animals are administered various levels (doses) of a suspected carcinogen, the observed responses (i.e., the percent of test animals developing cancer if the compound is carcinogenic) are usually in the range of about 5 percent to 95 percent. To observe responses at incidence rates less than about five percent requires large numbers of test animals. As will be seen, experiments designed to observe responses in the range of interest in establishing the no-residue standard, would require very large and often impractical populations of test animals. Therefore, the procedure of Mantel and Bryan,[1] and Mantel et al.,[2] as modified, is used to treat statistically the dose-response data from actual experimentation and to estimate the dose or level of the compound under test that would result in lifetime test-animal cancer rates no higher than a certain preselected rate.

Before discussing the many comments received on this feature of the proposal, the Commissioner reemphasizes that some operational zero must be defined if the no-residue requirement of the act is to be implemented. Regardless of the arguments for or against the Mantel-Bryan procedure, the Commissioner maintains that a procedure that takes into account the carcinogenic potency in test animals of residues (which the Mantel-Bryan procedures does) is far superior to any approach that fails to do so.

The modified Mantel-Bryan procedure described in the proposal was labeled excessively conservative by some comments and recklessly liberal by others. Those who considered the procedure too conservative objected to the proposed use of a series of conservative assumptions (shallow-slope, dose-response relations, low acceptable level of risk) and contended that any one of these assumptions alone could provide adequate protection to the public. Further, these comments argued that the practical application of the procedure has not been demonstrated, and suggested that it would prohibit the use of many valuable compounds. Persons who considered the proposed procedure too liberal objected to the proposed use of a lower confidence limit on the observed slope of the dose-response curve. Their objection is that the proposed statistical technique for extrapolating dose-response data obtained from animal tests seriously underestimates public risk. The technique provides a basis for establishing a dose level where there would be no significant human risk of cancer, thereby establishing a criterion for a residue detection method. Specifically, the comments contended that if the true statistics of the dose-response relation are logistic or linear, ex-

[1] Mantel, N. and W. R. Bryan, " 'Safety' Testing of Carcinogenic Agents," "Journal of the National Cancer Institute," 27(2):455-470 (1961).

[2] Mantel, N., et al., "Improved Mantel-Bryan Procedure for 'Safety' Testing of Carcinogens," "Cancer Research," 35:865-872 (1975).

trapolation with the slope of a probit transformation would seriously underestimate public risk. Further, these comments argued that the probit transformation leads to a paradox, in that strong carcinogens are treated less conservatively than weak ones. Regardless of their point of view, however, most of the comments supported the Commissioner's effort to elicit public discussion of the implementation of the anticancer provisions of the act.

2. *Choice of the Mantel-Bryan procedure*—(a) *Alternative statistical models.* Most of the comments favored the proposed adoption of the Mantel-Bryan procedure but without the modifications suggested in the proposal. A smaller number of comments recommended that a linear extrapolation would be a better alternative to the Mantel-Bryan procedure, and even fewer suggested the logistic or the angle distributions. Still other comments suggested that a comparative analysis of animal carcinogenesis data be required employing all alternative distributions and the smallest estimate of the "safe" level be used to define the no-residue standard for a compound. Finally, some comments indicated that, although the logistic and angle distributions have been used in biological sciences, there is no indication that either one provides advantages over the probit (Mantel-Bryan) or the linear distribution, and that, therefore, neither was appropriate for regulatory purposes.

Some comments favoring the Mantel-Bryan procedure argued that it has a theoretical rationale which is probably relevant to the carcinogenic action of chemical agents. A similar argument was made by some of the comments favoring the linear extrapolation. These comments also contended that the linear extrapolation has the public health advantage of being the most conservative of all procedures.

(b) *Limitations in available procedures and choice of procedure.* The Commissioner has extensively reviewed the known procedures that may be used to derive an operational definition of the no-residue standard of the act from animal carcinogenesis data. This review has persuaded him the same scientific and technological limitations are common to all. Specifically, because the mechanism of chemical carcinogenesis is not understood, none of these procedures has a fully adequate biological rationale. All require extrapolation of risk-level relations from responses in the observable range to that area of the dose-response curve where the responses are not observable. Matters are further complicated by the fact that the risk-level relations adopted by the various procedures are practically indistinguishable in the observable range of risk (5 percent to 95 percent incidence) but diverge substantially in their projections of risks in the unobservable range. Finally, the Commissioner concludes, no procedure is intrinsically more conservative than any other; the conservatism of any procedure depends entirely upon the restrictions and modifications imposed.

The comments failed to demonstrate that another procedure is superior to that of Mantel and Bryan [1] and Mantel et al.,[2] (Mantel-Bryan) and therefore the Commissioner has adopted it with some modifications. Moreover, the Commissioner concludes that some aspects of the Mantel-Bryan procedure offer distinct advantages over the other statistical procedures. It provides a clearly defined means for pooling data from multiple experiments and from multiple dose levels within a single experiment, thus permitting decisions based on the fullest use of available data. Further, the Mantel-Bryan procedure has a clearly defined mechanism for handling the spontaneous tumor rate. (See paragraph V.C.4.(d) of this preamble, below.) To overcome certain limitations of the Mantel-Bryan procedure, the Commissioner has adopted a number of modifications, which are described in § 500.87 and discussed in paragraph V.C.4 below in the preamble.

The Commissioner recognizes the significance of the decision to adopt the modified Mantel-Bryan procedure to implement the no-residue requirement at a time when that procedure, and similar procedures, as well as the relationship between test-animal experience and human risk, are under active and intense scientific study. He therefore has concluded that a review of this decision shall be undertaken in 2 years, and any appropriate modifications in the regulation will then be initiated.

3. *Time-to-tumor and other considerations.* Several comments contended that the proposal was deficient because it did not address the time-to-tumor aspects of chemical carcinogenesis. Some comments pointed out that Albert and Altshuler have developed preliminary statistical relationships between low levels of carcinogen exposure and time of tumor manifestation. It is the view of these authors that characterization of carcinogenic potential on the basis of incidence alone is not appropriate, because it ignores the life-shortening aspects of carcinogenesis.

The Commissioner generally agrees with these comments. He is faced, however, with a dilemma similar to that presented by the choice of statistical distributions. While statistical analyses based on incidence have been subjected to the scrutiny of use, the time-to-tumor relations developed by Albert and Altshuler have not. For this reason, the Commissioner concludes that the basis for extrapolation prescribed in the regulation shall be only incidence statistics, but the agency will initiate a review of the matter of time-to-tumor statistics in 2 years and consider the desirability and practicability of providing for their consideration.

One comment stated that "effects produced at higher dose levels * * * are useful for delineating the mechanism of action, but for any material and adverse effect, some dose level exists for man or animal below which adverse effects will not appear." The comment analyzed in detail the deficiencies of all statistical extrapolations and stated that approaches are available to define a true carcinogenic "no-effect" level. It contended that it is more appropriate to determine a biologically insignificant level using a safety factor based on competent scientific judgment.

The Commissioner disagrees with the contention that the classical toxicology concepts of "thresholds" and "biologically insignificant levels" are generally applicable to carcinogenesis. There is substantial scientific controversy over whether such concepts apply to irreversible processes, such as the chemical induction of malignant neoplasia. "Threshold" and "biologically significant level" concepts derive from short-term toxicity experiments which have no established meaning in biological processes that require long latent periods (up to 20 or 30 years) before lesion manifestation.

Several comments opposing the proposal suggested that the agency should maintain flexibility and evaluate the approvability of sponsored compounds based on assessments of benefit and risk, in effect offering another approach to establishing the operational zero for carcinogenic residues. The Commissioner concludes, however, that an approach that contemplates consideration of the benefits of use of a sponsored compound in defining the no-residue standard is incompatible with the anticancer provisions of the act.

4. *Modifications and restrictions on the Mantel-Bryan procedure*—(a) *Expression of dose level.* Several comments addressed the adjustments the Commissioner proposed to make in the "safe" level of Mantel and Bryan derived from the experimental animal data in order to establish an appropriate value for man. Some comments stated that adjustments for differences in food intake between experimental animals and man inappropriate when dealing with carcinogens. The comments stated that such adjustments would assume erroneously that all toxic materials have the same mode of action on a body weight basis. They further suggested that the relationship should be expressed in terms of concentration in the feed of the test animals and in the food of man when the diet in both cases is consumed ad libitum, other than on an amount-per-body-weight basis. Other comments argued that the conversion of animal data to man should be based on surface areas.

The final regulations specify that carcinogenicity tests shall be conducted with the test compound's concentration in the diet of the experimental animals held constant throughout the study. And the "safe" level derived from the modified Mantel-Bryan extrapolation of test-animal data shall be expressed as a concentration in the total diet (weight of compound/weight of total diet) of the animals and shall be directly applied at the "safe" level for the total diet of man. The Commissioner concludes that the arguments for conversion based on surface areas or on intake per unit of body weight have little basis. The comments

provided no evidence that these concepts are applicable to low-dose chronic exposures. The surface area concept is based on experience with short-term, high-dose studies. Furthermore, measurements of surface area are crude. Finally, surface area and body weight will vary, as will food intake per day, throughout the chronic study, thus requiring constant adjustments of dose.

Until evidence is compiled demonstrating that there is a more appropriate means of conversion from experimental animal to man with respect to chronic exposure and carcinogenic manifestation, the Commissioner will assume that the animal is the integrator throughout its lifetime of any observed response to a fixed concentration in the diet. The Commissioner has thus adopted the direct conversion approach (the "safe" level in parts per million, parts per billion, etc., of the diet of the experimental animals directly applied to the diet of man), which is the most conservative, as well as most practical, of the approaches considered.

(b) *Degree of data confidence.* The Commissioner disagrees with comments that characterized the proposal's requirement for 99 percent confidence intervals as another in a series of unnecessarily conservative assumptions. Confidence intervals characterize the quality of experimental measurement. The Commissioner concludes that a high degree of confidence should be demanded for decisions respecting carcinogens. He therefore has adopted the 99 percent level of confidence, and the final regulations require that all calculations based on experimental observations shall be made from or with the 99 percent confidence limits.

(c) *Slope used for extrapolation.* The proposal would have required that extrapolation be made with the lower 90th percentile of the observed dose-response curves. Numerous comments stated that the extrapolation should be performed with a slope of one, as proposed by Mantel and Bryan.

The Commissioner agrees with comments that suggested that use for extrapolation of the observed slope of the experimental dose-response curve could underestimate public risk, and has modified the regulation to call for a maximum slope of one. This requirement affords a high degree of confidence that, regardless of the actual configuration of the dose-response curve in the unobservable region, the maximum projected risk will be higher than the actual risk.

If the experimental dose-response curve exhibits a slope that is less than one, it is possible that this slope characteristic may also prevail in the unobservable region. To maintain the conservatism of the procedure, in such situations, the regulations require that the extrapolation be performed with the shallower slope. The Commissioner recognizes that there may be weak carcinogens whose actual dose-response curve slope may be relatively steep at the lower levels of response, with a plateauing (i.e., very shallow slope) in the experimentally observed region. In such a case, the procedure adopted would be ultraconservative. However, it is not possible to know the nature of the true slope in the unobservable region, and the agency must have a high degree of confidence that the maximum projected risk is above the actual risk.

(d) *Spontaneous tumor rates and data combination.* In the proposal the Commissioner recognized certain limiting features that are common to all extrapolation procedures, including that of Mantel and Bryan. These limitations concern the rate of tumor incidence in the control groups of animal bioassays and the selection or combination of data from different experiments. Since publication of the proposal, Mantel and coworkers[3] have developed procedures to deal with these issues. The Commissioner sees merit in these improvements and has adopted them in the final regulations.

In the original procedure published by Mantel and Bryan, the tumor incidence attributable to a given level of a chemical carcinogen was measured as the difference between the upper 99 percent confidence limit of the observed response of test animals and the lower 99 percent confidence limit of the observed response of control animals. The effect of this procedure on the derived "safe" level is minor when the tumor rate in control animals is low; however, when the control animals exhibit a high rate of spontaneous tumors, the effect of the procedure is far more pronounced. The improved procedure published by Mantel et al.[2] treats the rate of spontaneous tumors as an additional statistical parameter, which it is, and thus resolves this problem.

In many instances, the male and female animals of the same strain may exhibit significantly different responses to a compound. It is also apparent that the responses of different strains and species may be similar. It is always desirable to make maximum use of available information by appropriate combination of different data sets however, but prudence must govern the process of selecting and combining data. Combining different data sets increases the number of animals used in the analysis and therefore increases the confidence in the results. Yet, in many instances, different data sets contain different types of information. Mantel et al.[2] discuss the informational aspects of data combination with respect to pooling data from different experiments and from different doses. The Commissioner agrees in principle with most of their conclusions; nevertheless, he anticipates that situations will arise where the evidence in support of combining or not combining data will be equivocal. Therefore, he concludes that the statistical and biological evaluation of data will determine which data sets, if any, will be appropriate for pooling. Where there are significant statistical and/or biological differences in the observed responses, only subsets of data representing statistically and biologically compatible bioassays will be used for analysis.

(e) *Level of risk.* The proposal suggested that an accepted level of risk for test animals, and thus for man, could be 1 in 100 million. Many comments argued that this level of risk was unnecessarily conservative in light of the many other cumulative, conservative restrictions already imposed by the regulations. For the reasons set forth below, the Commissioner has concluded that this level of risk is unduly limiting without substantial compensation in terms of public health.

As the level of risk is decreased, the number of animals that are required in each test to bring the lowest limit of the assay's measurement derived from a no-carcinogenic-response test into the range of current analytical technology vastly increases. Thus, the time and resources that are necessary to plan, perform, and evaluate the test before submission to the agency in proper form increase enormously. This in turn increases the potential for interference from irrelevant variables or intervening forces. Then the amount of agency resources that must be committed to evaluate the data also increases almost geometrically. Finally, all these additional factors provide only a minor incremental increase in the degree of confidence in any decision that must be made on the results of these chronic toxicity tests. Consequently, the final regulations establish the maximum risk to be used in the Mantel-Bryan calculation as 1 in 1 million. The following clarifications of the meaning of the 1 in 1 million risk level demonstrate why the Commissioner believes that such a risk level can properly be considered of insignificant public health concern.

(i) The risk level of 1 in 1 million is a risk level for the entire lifetime of an individual.

(ii) This lifetime risk is the maximum, and therefore unlikely, human risk level. Because of the series of conservative assumptions built into the modified Mantel-Bryan procedure and into the derivation of the final "safe" level (see paragraph V.D., below in this preamble), the most likely human risk level will be several orders of magnitude less than this maximum.

(iii) The 1 in 1 million lifetime risk level assumes that an individual will consume maximum residue levels every day over a lifetime.

(iv) The use of this procedure for estimating acceptable level is based on the assumption that the only risk to the human population is that from residues of the sponsored compound, not from such intervening causes as disease or accidents (e.g., the average risk of fatality by motor vehicle accident per year is approximately 1 in 4,000). Because the population is constantly at risk from a wide range of factors, however, any increment of increased risk associated with exposure to residues of multiple compounds is at most in the vanishingly small range.

D. DERIVATION OF THE LEVEL OF TOTAL RESIDUES OF CARCINOGENIC CONCERN WHICH CAN BE TAKEN AS SATISFYING THE NO-RESIDUE REQUIREMENT OF THE ACT

As explained in the previous section, a potential residue level corresponding to a risk of 1 in 1 million in test animals (i.e., the "safe" level derived from the modified Mantel-Bryan procedure) can be considered the level that represents no significant carcinogenic burden in the total diet of man. This level is assigned in the final regulations the symbol S_0 and, expressed as a fraction in the total diet (i.e., parts per billion, parts per trillion) of the test animals, shall be directly taken as the potential undetected residue level that is safe in the total diet of man.

In some cases, residues in addition to the sponsored compound itself will have been selected for carcinogenicity testing. In these instances, "safe" levels will be derived for each of the compounds that have undergone testing. The compound exhibiting the lowest value for the "safe" level is the most potent carcinogen of those tested and constitutes the greatest potential carcinogenic threat among the residues. The Commissioner will, accordingly, choose the smallest value of the various "safe" levels, assign to it the symbol S_0, and assume that it represents the potential carcinogenic burden that may result from the administration of a sponsored compound to food-producing animals. Additionally, because other tested residues may have exhibited carcinogenic properties (albeit less potent) and still other, untested residues may represent carcinogenic risks, the S_0 will be taken as the sum of the levels of all of the residues. Potential residues in the total human diet cannot exceed S_0 if that diet is to bear no significant carcinogenic risk to man. The only residues that can be excluded from the sum of residue levels are those that have been unambiguously shown to be noncarcinogenic.

Although it will already be apparent to the attentive reader and to the trained scientist, it bears reiteration at this point that S_0 (or any figure derived on the basis of adjustments described below) does not represent a level of residues "approved" for introduction into the human diet. The purpose of these regulations is to establish criteria for the evaluation of assays for the measurement of carcinogenic animal drugs. These criteria must include some lowest level of reliable measurement that an assay is required to meet. In defining a level of potential residues that can be considered "safe," therefore, the Commissioner is establishing a criterion of assay measurement that, if it can be met for a compound, will assure that any undetected residues resulting from the compound's use will not increase the risk of human cancer.

E. CORRECTIONS FOR FOOD INTAKE

Several comments argued for and others opposed further adjustments based on patterns of food consumption. Some comments contended that the "safe" level of Mantel and Bryan in the animal diet should be directly applied as the upper allowable limit in man's diet and in any component food in the human diet. These comments argued that this limit should not be raised by consideration of intermittency of consumption of particular foods or of the proportion of the total diet represented by an individual food. They suggested that individuals who consume above average amounts of food would be exposed to above average, and thus possibly harmful, levels of residues. Further, these comments contended that the act does not provide a distinction between people who consume average diets and people who consume above-average quantities of exotic foods; both groups are entitled to equal protection. They argued that adjustments for exposure frequency based on food consumption patterns assume that continuous long-term exposure to a carcinogen precedes the development of cancer.

Many other comments urged that adjustments should be made based on the proportion of the specific food in the total diet and the frequency of exposure. These comments generally favored the use of food consumption data, so that the degree of conservatism was more uniformly applied taking into account the relationship of the particular food to the total diet.

The Commissioner disagrees with the contention that no adjustments should be made for factors of exposure. Section 512(d)(2)(A) of the act requires the Commissioner to consider the probable consumption of a drug and of any substance formed in or on food because of its use. Analysis of carcinogenesis data provides S_0. The no-residue standards of the act has been defined as satisfied when the sum of the levels of all potential undetected residues of the sponsored compound (excluding only those that have been found to be noncarcinogenic) would not exceed S_0 in the total diet of man. Because products derived from food-producing animals do not constitute the total human diet, it is therefore appropriate that S_0 be corrected for probable human consumption of specific tisues. The Commissioner agrees, however, that any adjustments must be conservative to assure that all segments of the population are protected.

The Commissioner has consulted available data on food consumption patterns in the United States, and concludes that muscle tissue and eggs can be considered, conservatively, to each constitute one-third of the total daily human diet. Since milk can constitute the total daily diet of any individuals (e.g., infants), no adjustment will be made for this commodity. Adjustments for frequency of exposure for tissues other than muscle, milk, or eggs (i.e., kidney, liver, etc.) will be considered only if the proportionate levels of potential undetected residues in such other tissues, compared to muscle, are such that intake of muscle tissue on days when other tissues are not being consumed provides an insignificant contribution to the total exposure to residues (i.e., S_0 is never exceeded in the total diet of human beings).

The final regulations use the symbol S_m to represent the level of total residues of carcinogenic concern that can be operationally defined as satisfying the no-residue requirement of the act for specific tissues. If, for example, a particular animal drug used in cattle were found to have an S_0 of 10 parts per trillion, the assay required for approval of the drug would have to be capable of reliably measuring residues of 30 parts per trillion and above in muscle tissue.

F. OTHER POSSIBLE ADJUSTMENTS

Several comments urged that the regulation should not provide for adjustments for the degradation of residues in food under normal conditions of storage and cooking. Others suggested that such data should not be required but should be taken into account when available. Still other comments expressed the fear that such data would be used to dilute the conservative intent of the regulation; they argued that the term "normal condition of storage and cooking" would be difficult to define, and it might reduce protection in situations where actual storage and food preparation practices did not approximate experimental conditions. Finally, some comments suggested, generally, that such studies should be required only when there is reason to believe that such information would assist in protecting public health.

The Commissioner agrees that the parameters appropriate to such studies have not been defined, and he has deleted from the final regulations references to postslaughter residue degradation studies. When there is reason to believe that storage conditions or food preparation methods might lead to the formation of potentially toxic residue products, however, the Commissioner will require appropriate special investigations. Petitioners are encouraged to explore the postslaughter stability of residues. Experience has shown that residue stability can be a complicating factor in studies for the validation of assays for dosed tissues. The Commissioner encourages research in this area but until appropriate information can be reliably incorporated in the food safety decisions, such data will not be used to liberalize the requirements of the regulations.

G. CONSIDERATION OF OTHER RELEVANT SAFETY FACTORS

Originally, the Commissioner proposed that the Mantel-Bryan calculation be modified to account conservatively for drug use patterns, e.g., the administration of a drug in the treatment of diseased animals. Comments demonstrated that disease incidence does not occur randomly within a geographic area or within specific animal groups. Although a disease may have an overall incidence of only 10 percent, the affected group may be located in a single area. Therefore, the Commissioner is unable to conclude that evidence exists, or other safety factors are available, to permit him to calculate the effect of such drug usage, and he has deleted this provision from the regulation.

VI. Metabolic Study to Select Marker Residue and Target Tissue

A. THE CONCEPT

Before he can approve the use of a sponsored compound, the Commissioner must assure that a practical and reliable assay is available that can measure carcinogenic residues at the level which discriminates safe from unsafe food, i.e., the assay must be capable of determining when S_m is exceeded in each edice tissue. One approach to this problem would be to require assays that can be used to measure every residue in each of the various edible tissues. Because the number of residues in edible tissues and the number of tissues can sometimes be large, it is unlikely that such an approach could be put to practical use. The Commissioner has determined that another approach is possible that is far more practicable and sacrifices no principle of safety. This alternative approach centers on the concepts of a marker residue and a target tissue.

A marker residue is a residue whose level in a particular tissue is in a known relationship to the level of the total residue of carcinogenic concern in all edible tissues and which, therefore, can be taken as measure of the total residue of interest in the target animal. Once a marker residue is selected and its quantitative relationship to the total residue is determined, it is possible to calculate a level, for purposes of these regulations, R_m, which is that level of the marker residue that must not be exceeded in a selected tissue (the target tissue) if the total residue of carcinogenic concern in the edible tissues of the target animal is not to exceed S_o. The marker residue can be the sponsored compound or any of its metabolites, or a combination of residues for which a common assay can be developed.

The target tissue is that tissue in which the absence of the marker residue at R_m or above can be taken as confirmation that the safe residue level, S_m, is not exceeded in any of the edible tissues. When a marker residue and a target tissue are selected, a practicable assay must be developed that can reliably measure the marker residue in the target tissue at levels at least as low as R_m, and conditions of use of the sponsored compound must be established that assure that, in practice, the potential marker residue level in the target tissue does not exceed R_m.

When it is determined, using as assay demonstrated to be capable of reliably measuring the marker residue in the target tissue at levels at least as low as R_m, that there is no such residue at levels at or above R_m, it can be concluded that the no-residue standard of the act has been satisfied for all edible tissues in the animal under examination. Conversely, if the market residue is found in target tissue at levels equal to or greater than R_m, all edible tissues must be considered unsafe for human consumption.

B. APPLICATION: DATA DEVELOPMENT AND CALCULATION OF R_m

1. *Marker residue.* Application of the concepts of marker residue and target residue requires an experimental determination of the quantitative relationships of residues that might serve as markers (including any which have definitely been shown to be noncarcinogenic, since theoretically one of these might be selected as marker residue) to the total residue in each of the various edible tissues which might serve as target tissues. Further, because these relationships change with time, the levels of potential marker residues in the potential target tissues must be measured over time, and tissue concentration-time profiles must be constructed. These depletion profiles will be derived from measurements made in target animal tissues after cessation of exposure to the sponsored compound. Finally, because the results of carcinogenicity testing have been used to set limits for total potential undetected residues in each of the individual edible tissues, the depletion profiles must include measurements of the total residue in each potential target tissue to levels at least as low as the S_m appropriate to the tissue. Additionally, depletion profiles for one or more potential marker residues must be constructed and include measurements of levels of residues corresponding to the times when the total residue has reached S_m (Plates I and II set forth in § 500.89 (31 CFR 500.89).)

Part III of this preamble describes the requirements for the study of the metabolic fate of a sponsored compound in target animals. Although the purpose of this earlier metabolic study is to provide information for selecting residues for carcinogenicity testing, the same principles and requirements are applicable here and must be followed in acquiring the information necessary to construct depletion profiles. However, to meet the depletion profile requirements prescribed by the regulations, a second metabolic study of the sponsored compound in the target animals may be necessary. This second and possibly more refined study may require the use of a larger number of animals, for it will be necessary to determine the total number and the quantities of residues, not only at two points in time, but at several appropriately spaced time intervals starting immediately after cessation of exposure and continuing until the residues in each of the potential target tissues has reached a level corresponding to a total residue level of the appropriate S_m (e.g., for meat, milk, or eggs). If the initial metabolic study were done with the degree of precision required to select a marker residue and a target tissue, of course, it need not be repeated.

Selection of a marker residue will be based on examination of depletion profiles. Generally, there will be some time at which the sum of the levels of the individual residues of carcinogenic concern will fall below the S_m appropriate to the tissue under examination. Residues that are potential markers will be present at a known concentration (R_m) at this same time (T_L of Plate I), and in a definite (although perhaps rapidly changing) quantitative relationship to the total residue (Plate II).

With the quantitative relationships established, it will be possible to select one of the residues as a marker. Ordinarily, the residue selected will have the following characteristics: (i) It will represent at least 10 percent, and usually a great deal more, of the total residue burden at the time when the total residue was depleted to S_m; (ii) it will be stable, easily isolated and characterized, and susceptible to manipulation for assay development and implementation; (iii) it will be undergoing relatively rapid change in concentration at the time the total residue burden is at or near S_m (i.e., a change in its concentration will be a sensitive indicator of the time when the total residue burden has depleted below S_m. While other considerations may enter into the selection of a marker residue, these three will ordinarily be most important.

There may be instances in which no single residue can adequately fulfill the requirements which a marker residue must meet. In such instances, it may be necessary to select some combination of residues which, taken together, can represent the total residue burden. It should be noted that a marker residue can be a compound which is not a carcinogen, but is an unambiguous indicator, in the manner already described, of the presence or absence of carcinogenic residues.

2. *Target tissue.* Selection of a target tissue requires a comparison of the depletion profiles for each of the edible tissues (Plate I set forth in § 500.89). A target tissue will be selected based on assurance that the absence of the marker residue at or above R_m assures that carcinogenic residues are absent from the slowest depleting tissue, and thus that the entire animal is free of carcinogenic residues.

When a compound is to be used in milk- and egg-producing animals, milk and eggs will be target tissues in addition to one tissue selected as the target tissue to represent the depletion of residues in all of the edible carcass. In such cases, it may be necessary to select a marker residue for milk or eggs that is different from the marker residue selected for the target tissue representing the edible carcass.

3. *Calculation of R_m.* The level of the marker residue which is present in the target tissue at the time (T_L) when the sum of the levels of the residues in the slowest depleting tissue (excluding any residues that have definitely been shown to be noncarcinogenic) is equal to S_m for that tissue, is the R_m for that marker residue. The depletion profiles will be used to select R_m (Plate II set forth in § 500.89).

For example, assume (i) that liver is the target tissue of animal drug, P, intended for use in cattle; (ii) that the

only residues of P are the parent compound, P, and a metabolite P_1; (iii) that T_L is 3; (iv) that S_m for the sponsored compound is 29 parts per trillion; and (v) that the following is a chart of the depletion profile of the drug.

[In parts per trillion]

Time	Total residue burden	P	P_1
0	100.0	75.0	25.0
1	65.4	44.6	21.8
2	42.0	25.3	17.3
3	29.0	15.0	14.0
4	21.0	9.0	12.0
5	15.0	5.0	10.0

In this case, before the drug can be approved for use, the petitioner must develop an assay that will satisfy the evaluation criteria in liver for either P at least as low as 15 parts per trillion or P_1 at least as low as 14 parts per trillion. Because P is depleting faster than P_1, when the total residue burden is 29 parts per trillion, P may be the preferred compound to select as the marker residue since it does provide a more accurate assessment of when the total residue burden reaches 29 parts per trillion (S_m). Another example is provided in Plate II in § 500.89.

VIII. Sponsored Compounds Affecting Pools of Carcinogenic or Potentially Carcinogenic Substances Endogenous to Target Animals

A. Applicability of No-Residue Requirement

The act requires that in making food safety decisions, the Commissioner take into account all substances formed in or on food by the administration of sponsored compounds to food-producing animals. It is well recognized that: (i) Several substances endogenous to food-producing animals are suspect or proven carcinogens; (ii) in any given animal species or breed, the size of pools of such endogenous substances vary widely with such attributes as sex, age, lactation, state of estrus, pregnancy, geographic location, and animal husbandry practices; and (iii) man has had sustained exposure to such endogenous substances for centuries. Whether normal levels of human exposure to these substances are responsible for human carcinogenesis is unknown, but the Commissioner maintains that the use of drugs that can cause an increase in human exposure to such compounds has the potential of increasing the risk of human carcinogenesis. The use of such drugs must therefore be controlled.

In dealing with potentially carcinogenic endogenous compounds, the proposal declared that the intent of the no-residue requirement of the act is the maintenance of the normal human dietary content. Thus, the regulations require the determination of the effects of sponsored compounds on the normal background levels of potentially carcinogenic endogenous compounds. If a compound is found to increase such levels, conditions of use must be established so that normal background levels are not exceeded in the animal when the animal is slaughtered. The regulations also require the development of practical assays for measuring endogenous compound levels.

Several comments on this segment of the proposal expressed concern over the meaning of the term "endogenous compounds" and questioned how such compounds are to be distinguished from "exogenous compounds." Others questioned whether the former term includes chemical derivatives (estradiol benzoate) of bona fide endogenous compounds (estradiol) or essential nutrients (some amino acids, minerals, vitamins). Comments also expressed doubt about the distinction between endogenous and exogenous compounds in cases where the administered compound can be metabolized to residues of both classes. Some comments also argued that all externally administered compounds should be considered exogenous, as the true meaning of the term implies.

Other comments suggested that endogenous substances of interest be subjected to toxicological testing and tolerances be set if such substances are found to be not carcinogenic. Some expressed doubt that available technology could meet the requirements of the proposed regulation. They contended that the terms "normal conditions of use" and "normal background levels of endogenous compounds" would be either extremely difficult or impossible to define. The Commissioner recognizes the difficulty of the task, but concludes that administered compounds that increase the naturally occurring level of potentially carcinogenic endogenous compounds present special problems of control which the regulation must address and resolve.

B. Definitions

An endogenous compound is any compound that is metabolically produced by and is present in untreated target animals. Any sponsored compound that is found to increase the normal background levels of a potentially carcinogenic endogenous compound shall be subject to these regulations regardless of how the increase is brought about.

For instance, estradiol benzoate, which is by the above definition clearly not an endogenous compound, is metabolically converted to the endogenous compound, estradiol, and may thus cause an increase in normal background levels of that substance. Estradiol may itself be administered, possibly again causing target animal pools of estradiol to increase above background. Finally, a sponsored compound may indirectly cause an increase in tissue levels of estradiol by affecting any number of hormonal regulatory systems in the target animals. While in each of the above cases the cause of the increases in normal background levels of estradiol was different, the result was the same. And it is the result that must be monitored and controlled. It is thus of little use to distinguish between "endogenous" and "exogenous" sponsored compounds. Rather, it is useful only to distinguish between administered compounds that can cause changes in normal background levels of potentially carcinogenic endogenous compounds, which can unambiguously be defined, and those administered compounds that do not affect such levels.

Essential nutrients are not included in the definition of the classes of compounds that will be regulated by these regulations. In a strict sense, essential nutrients are not endogenous. Although present in the tissues of animals and required for growth and health, they are not produced by the animals and must be supplied from external sources. These features place essential nutrients in a distinct class of "required exogenous compounds," which must continue to be regulated in a unique manner. Determination of the allowable use of essential nutrients must reflect the nutritional requirements of the target animals. When used according to label directions, essential nutrient supplements should restore but must not exceed the essential nutrient levels found in natural foods adequately sustaining normal growth of healthy animals. Furthermore, the levels of animal essential nutrients found in human food derived from supplemented animals must not exceed the levels in food derived from normal healthy animals fed a nutritionally adequate natural diet.

C. General Procedures

If available information shows a sponsored compound might affect pools of potentially carcinogenic endogenous substances in target animals, and cause an increase in the level of such substances above the level considered to be safe by the criteria of these regulations, the petitioner shall be required to demonstrate whether or not these suspicions are true. The need for, and the depth and breadth of, studies required to demonstrate this effect must be specified on a case-by-case basis.

The procedure required is fourfold: (i) Establishment of normal background levels (or "norm") of the endogenous compound of carcinogenic concern in the target animals; (ii) determination of the effects of the sponsored compound on the norm; (iii) establishment of safe conditions of use of the sponsored compound by demonstrating how the compound can be used in a way that assures that the norm is restored in the target animals before slaughter; and (iv) development and validation of a practical assay to measure the endogenous compound at levels determined to be normal. The regulations specify how each of these steps is to be accomplished.

D. Specific Steps Required

The petitioner shall first be required to determine experimentally the normal background levels, or norms of the potentially carcinogenic endogenous compounds of concern in untreated target animals. A norm must be specific for the target animals and for the intended conditions of animal husbandry, and must include the effects of age, sex, breed, and geographic location. The sponsor shall

provide the norm in the form of a curve of cumulative frequency distribution of untreated target animals over the observed levels of the endogenous compound. The curve shall also include 99 percent confidence bounds (Plate III appearing in § 500.89).

The median and shape of the frequency distribution must be known so that shifts in the norm can be measured. For this reason, the assay used to determine a norm must yield values for the endogenous compound different from zero for at least two-thirds of the untreated target animals. This latter requirement will permit calculation of the median and frequency distribution with a high degree of reliability, while recognizing the practical limits of technology. Morever, because the area of interest is that around the median, the requirement does not compel the petitioner to gather unnecessary data since the values at the lower end of the distribution are irrelevant.

The sponsor shall then determine the effects of the sponsored compound on the norm, and shall provide data on the postexposure decay of any observed increases in the norm. The norm shall be considered restored when the distribution of values for the endogenous substance of concern observed in a group of treated animals is with 99 percent confidence the same as the norm.

The norm, as defined, takes into account those variables that affect background levels. The final regulations thus attempt to respond to those comments suggesting that "normal background levels" would be difficult to define.

E. ENDOGENOUS MARKER RESIDUE; CALCULATION OF R_m

If the norm of an endogenous substance of carcinogenic concern can be increased by the administration of a sponsored compound, the endogenous substance can become an endogenous marker residue, i.e., its presence above certain levels can be considered an indicator of potentially carcinogenic residues in food. Approval of the use of such a sponsored compound shall be contingent upon the petitioner's furnishing data demonstrating that the norms are restored in the target animals before slaughter, and upon the availability of a practical assay that can reliably measure the endogenous marker residue in target animals. Such a regulatory assay must be capable of measuring the marker residue at the level, R_m, corresponding to the 33d percentile of the norm (Plate III set forth in § 500.89).

The R_m for an endogenous marker residue derives from an entirely different conceptual approach to safety than that used for the derivation of an R_m for an exogenous marker residue. To monitor shifts in the norm, the Commissioner must be able to measure the median and to determine the shape of the distribution. An assay capable of measuring the 33d percentile of the norm, and levels above this, provides the required analytical capability. The same assay evaluation criteria apply to endogenous compounds as to other compounds covered by these regulations.

Accordingly, the Commissioner has revised the regulations, which as proposed, would have established the lowest limit of reliable measurement at the 99th percentile of the norm. As the comments noted, an assay that can measure only the upper 99th percentile would not be able to detect many shifts in the norm, which is its primary function. The final regulations require an assay capable of a lowest limit of reliable measurement of the 33d percentile of the norm, which will readily detect any shifts in the median or mean of the norm. Actual monitoring, which is performed by the Animal Plant and Health Inspection Service of the United States Department of Agriculture, may occur at or above the 50th percentile of the norm but such monitoring will detect violative residues and detect significant shifts in the norm.

F. ALTERNATIVE PROCEDURE

Comments contended that an alternative to the foregoing procedure should be available for regulating endogenous substances. It was suggested that a tolerance for an endogenous compound can be established, even at levels above the norm, provided appropriate toxicity testing on the compound is carried out and a safe level can be established in accordance with parts IV through VII of the preamble and §§ 500.84 through 500.90 (21 CFR 500.84 through 500.90). Separate mechanisms with distinctly different rationales have been developed to measure compliance with the no-residue standard of the act for endogenous and exogenous compounds.

As noted earlier, for exogenous compounds the regulations require development of an assay with a minimally acceptable lowest limit of reliable measurement at or below the level needed to assure that any undetected residues pose essentially no increased risk of cancer in the population. Moreover, should a new assay with a lower limit of reliable measurement be developed at a later time that will satisfy the essay evaluation criteria, that assay will be adopted by the Commissioner. On the other hand, the method for measuring compliance with the no-residue standard for an endogenous substance is based on calculation of the norm, a calculation that is independent of and probably unrelated to the lowest limit of an appropriate assay's reliable measurement. The Commissioner concludes that monitoring of changes in the norm is the best available method for regulating the use of compounds that may increase pools of potentially carcinogenic endogenous substances, and rejects the suggestion that a tolerance for such compounds be established. The Commissioner would be receptive to suggestions for alternative mechanisms of control, but until an acceptable alternative is identified, all such compounds will be required to comply with the requirements imposed by §§ 500.89 (c) through (e) and 500.90.

VIII. REGULATORY ASSAY: EVALUATION CRITERIA AND APPROVAL PROCESS

A. INTRODUCTION

The Commissioner can approve a sponsored compound for use in food-producing animals only if the intended use of the compound does not result in the accumulation of potentially carcinogenic residues in edible tissues and if an assay is available that can reliably measure such residues at and above the R_m. The assay must also be suitable for monitoring food from animals administered the compound to prevent food from reaching the marketplace if it is adulterated with potentially carcinogenic residues resulting from misuse of the compound.

Several comments argued that the proposal would discourage the search for better assays, and that this was not in keeping with the intent of the cancer provisions of the act. Further, some comments contended that FDA should only be concerned with the approval of assays that avoid false negative results and that any detected residue should be investigated to determine its identity. Other comments proposed that when more "sensitive" assay methods (i.e., assays with still lower limits of reliable measurement) are developed, the assays should only be used as screening tests and that the required "sensitivity" (or safe level) derived from the statistical analysis of animal carcinogenesis data should be retained for regulatory action. These comments argued that unless new biological information warrants a change in assay "sensitivity," new regulatory assays should not be adopted. Comments stated that the efforts to increase "sensitivity" had to be balanced by the need to assure the practicability of an assay for regulatory use, the desirability of avoiding false negatives, and the importance of reproducibility of results. These comments implied that, given these countervailing concerns, more "sensitive" assay methods should not be adopted because the proposed statistical treatment of carcinogenesis data is sufficiently conservative to protect the public health.

Still other comments suggested that more practical methods should be approved for purposes of screening which would accept a low level of false positives with a high degree of assurance that false negatives would not occur. Confirmatory methods, which would undoubtedly require more time for cleanup of samples and greater instrument specialization, should then be used to provide evidence that can withstand legal scrutiny. Some comments stated that certain reagents and instruments required for an assay may not be readily available because of their unique applicability. They suggested that the regulation be changed to allow sponsors to supply such items when necessary. One comment pointed out that the word "control" in the phrase "well-equipped analytical control laboratory" connotes a highly specialized laboratory which is unlikely to have the necessary instrumentation for residue analysis, and hence urged that it be deleted.

Because the assays required by these regulations are to be used for regulatory monitoring of residues of potential carcinogenic concern in human food, the Commissioner concludes that rigorous criteria must be established for approval of these assays. Furthermore, a proposed assay must be subjected to an objective evaluation to determine if it meets the criteria. Only then can the Commissioner assure that an assay will provide a reliable and practical monitoring device to prevent violative residues in food. Many comments in essence contended that more explicit criteria and evaluation procedures should be specified, and the Commissioner concurs with these comments.

Any assay is characterized by a set of attributes which determine its quality: dependability, practicability, specificity, accuracy, and precision. These regulations specify objective criteria for these attributes. A proposed assay must be shown to meet these criteria during study in a single laboratory and also in interlaboratory study in government regulatory laboratories. The latter requirement is essential, because the assays are to be used in several regulatory laboratories (FDA, USDA, and State laboratories), and the Commissioner must determine in advance that an assay will perform in more than one such laboratory. The regulations specify that the interlaboratory validation study shall be carried out in those laboratories (USDA and FDA) that will be using the method in surveillance and enforcement programs.

The steps in obtaining approval of an asay are: (i) Assay development and study by the petitioner to determine if the assay satisfies the acceptability criteria; (ii) FDA review of the petitioner's study to determine suitability of the assay for evaluation in interlaboratory study; and (iii) interlaboratory validation study, again approval contingent upon satisfaction of acceptability criteria.

B. SOURCES OF DATA TO SUPPORT THE ASSAY

Data from studies of an assay using three types of samples are necessary to support approval. The petitioner must prepare samples of target tissue to which known amounts of marker residue are added ("spiked" tissues), and compare responses obtained from assays using these tissues with responses obtained from assays of target tissues known to be free of marker residues (control tissues). In constructing an analytical curve from these data and determining its 99 percent confidence limits (plot of observed response versus concentration of marker residue), as many samples as possible should be run, preferably by different analysts, for interlaboratory validation of the assay will eventually be required. The variability among different analysts can be determined at the developmental stage and adjustments made before the assay is submitted for FDA review.

A petitioner should also be satisfied that the assay meets all of the evaluation criteria and also that it is consistent with general principles of good analytical practice before submittal for FDA review. Past experience shows that a petitioner's failure to follow good analytical practices during initial assay studies often results in interlaboratory failure even though the initial results may appear satisfactory during a paper review of the assay by FDA. A petitioner should assure that no results enter the construction of an analytical curve when it is known that the results were obtained using other than acceptable principles of analytical practice.

In addition to the spiked tissue tests, a petitioner must also submit data showing the applicability of the proposed assay to target tissues taken from target animals treated with the sponsored compound ("dosed" tissues). To validate the assay, dosed tissue samples are required that contain the marker residue at a level approximating R_m. A standard curve must also be submitted, constructed by taking the marker residue of known purity at different concentrations, determining the response, and plotting the relationship.

C. SUBMISSION OF DATA

Agency resources for reviewing and validating assays are limited. The Commissioner therefore has established a precise format for submitting the data to support acceptance of an assay. It is a well-recognized principle, applied both by the courts and administrative agencies, that a standard format can be required for pleadings, requests for licenses, and other applications. This format may also designate special types of information that must be contained in the submission. Therefore, the agency will refuse to accept a petition or review an assay when the request for approval fails to conform to the format outlined below.

1. *Assay description and petitioner's evaluation.* The petitioner must provide a complete description of the assay to allow FDA to determine whether it is potentially acceptable. Because this threshold determination of acceptability will trigger an extensive interlaboratory validation procedure, the Commissioner concludes that the discussion must be sufficiently rigorous to minimize waste of agency resources. Therefore, the submission must discuss in detail:

(a) What equipment and reagents are necessary;

(b) How the assay is performed; and

(c) How the assay complies with the dependability, practicability, specificity, accuracy, and lowest limit of reliable measurement criteria prescribed in § 500.90(d) (21 CFR 500.90(d)) and discussed under paragraph VIII. E. below in this preamble.

2. *Data.* The data and worksheets, including spectrograms, chromatograms, etc., from the spiked tissue, dosed tissue, and control tissue analyses are also necessary for the preliminary review of the assay to determine whether it actually complies with the evaluation criteria.

D. FDA REVIEW

The Commissioner will conduct a paper review of a petitioner's submission to determine whether an assay complies with the acceptability criteria. These regulations generally alert potential petitioners to the applicable statutory standards and criteria, which should permit a petitioner to assess preliminarily the acceptability of an assay before filing a petition, and thereby reduce the agency's workload.

If on preliminary review an assay appears to comply with the evaluation criteria, it will then be subjected to the interlaboratory assay validation study to determine whether it is indeed a practicable and reliable regulatory tool. Should the initial review establish that the assay fails to meet these criteria, the petition will be denied. A conclusion that an interlaboratory assay validation study should be initiated, however, in no way guarantees that a proposed assay will be eventually approved.

E. ASSAY ATTRIBUTES AND ACCEPTABILITY CRITERIA

An assay must meet the following attributes and criteria:

1. *Dependability.* Dependability is the attribute denoting the likelihood that the proposed assay will yield no result because of uncontrollable features inherent in its design. Almost all assays will, on occasion, fail to yield any result. Often this occurs because of mishandling by the analyst, but sometimes failure may be the result of some aspect of the assay itself that may have been inadequately studied and defined or that cannot be controlled. For example, assays depend upon the availability of a standard against which measurements are compared. If the integrity of the standard depends on certain environmental factors (e.g., purity of the solvent in which it is maintained, temperature, light intensity, etc.) and these factors are understood, it may be possible to prevent assay failure. If this dependence is not known, however, the assay may fail and, depending on the sensitivity of standard integrity to the environmental factor of importance, may fail often. In this example, failure can mean a highly inaccurate result, assuming some fraction of the standard's integrity is retained, or it can mean no result at all, assuming complete loss of integrity.

The Commissioner concludes that assays used to monitor carcinogenic residues in food must be free of such uncontrollable features, and failure of a proposed assay to yield results during the petitioner's assay development studies or interlaboratory validation study can be a cause for refusing to accept the assay and for denying the underlying petition. Accordingly, the regulations require a petitioner to record and furnish all the information on, and provide an explanation of, runs of the developed assay that are begun, but never finished.

2. *Practicability.* The regulation under § 500.90(d)(2) defines the practicability attribute as follows:

The assay shall be considered practicable only if it is suitable for routine use in a government regulatory laboratory. The time required to complete the assay must be consistent with regulatory objectives (monitoring, compliance, etc.). All supplies, equipment, reagents, standards, and other materials necessary to conduct the assay must be commercially available except that reference standards may be supplied by the petitioner if they are not commercially available. The Commissioner will withdraw approval of any assay method and initiate regulatory action against the sponsored compound, if the petitioner breaches such a condition of the compound's approval.

An assay must possess no characteristics that may counteract the purpose for which it is developed. Accordingly, the Commissioner has established criteria for practicability in terms that relate specifically to the nature of the laboratories in which the assay will be used (i.e., regulatory laboratories where time and availability of equipment and reagents are critical factors in their ability to perform satisfactorily the mandated functions).

The inability to use an assay at a regulatory laboratory because a needed reagent is not readily available or because excessive time is required to complete the assay presents potential risks to public health and cannot be sanctioned. Obviously, some assays will require some unique items, particularly reference standards. The Commissioner agrees with comments suggesting that as long as a sponsor makes reference standards available to all persons having an interest, the requirements of the regulation will be met. A commitment to supply reference standards when they are not commercially available may be made a condition of the sponsored compound's approval, and failure to supply the government or other laboratories as required is a basis for withdrawing a compound's approval. The Commissioner concludes that an assay is not practical if it is dependent on the use of any other unique equipment or materials that are not commercially available.

3. *Specificity.* The regulations specify that for an assay to be accepted, an observed response must without question be due to the compound being measured and that compound only. It is a fundamental part of the development of an assay to determine whether or not it possesses this important attribute. Among analytical chemists and biochemists, an "assay" that does not demonstrate this attribute is of little value, and indeed, in a regulatory setting, such an assay could be dangerously misleading. For this reason, the Commissioner has established rigorous specifications for this attribute.

In general terms, specificity describes the uniqueness of the relationship between the observed effect (or response) and the applied stimulus (in this case the chemical under analysis). In analytical chemistry and biochemistry, the term specificity is commonly used in reference to the uniqueness of a response resulting from the application of a stimulus having specific charactertistics; that is, the term has a qualitative dimension only in that it does not relate to either the quantity of response or stimulus or to the nature of the relationship between response and stimulus. Both of the latter criteria, which might also be considered aspects of specificity, are central to good analytical practice. The regulations consider both the qualitative and quantitative aspects and groups them together under the general attribute of specificity. The Commissioner's objective is to assure that, whatever the observed response, it is uniquely related to the marker residue both qualitatively and quantitatively. The establishment of an analytical curve (not simply a standard curve, but one derived from actual measurements obtained on tissue samples containing known amounts of marker residue at different levels and from control samples) provides the means to determine whether the responses produced by an assay are single-valued, as they must be if an assay is to be considered fully specific. Only assays that yield continuously increasing or decreasing analytical curves will satisfy the criterion of single-valuedness.

Finally, the regulations require that the assay contain a sufficient number of independent measurements utilizing independent physicochemical principles to assure specificity (i.e., the identity of the marker residue must be confirmed). There may be many ways in which specificity can be demonstrated experimentally. A petitioner may use highly sophisticated research tools to demonstrate that a proposed assay is specific in the senses discussed above. However, a regulatory analyst, using an approved assay, must have at his disposal some technique (again capable of meeting other criteria) which can provide assurance that an observed response is due to the marker residue. At present, mass spectroscopy is probably an ideal choice for acquiring the requisite specificity, although there are other possibilities. Some determinations (e.g., those requiring enzymes) may have an inherent high specificity, but others have low specificity (e.g., gas, thin-layer, and liquid chromatography) and require other, independent, types of measurements to achieve the requisite confirmation of identity. By adopting this definition of specificity, the Commissioner concludes that all concerns expressed in the comments over "false positives" or "false negatives" are moot.

4. *Accuracy.* Assays yield measurements of concentration that are in some proportion to the true concentration of the compound being measured. The accuracy of an assay is expressed as a percent of the compound's true concentration. The regulations prescribe a specific accuracy criterion: The averages of the observed responses must fall within 60 to 110 percent of the true value. The criterion is consonant with current, good analytical practice and is based on agency experience with methods that are routinely used for trace analysis.

5. *Lowest limit of reliable measurement* (L_m). To be accepted for regulatory purposes, an assay must be able to distinguish, with a very high degree of certainty, target tissues that contain levels of the marker residue at or above R_m from target tissues that do not. This distinction must be reproducible and capable of supporting legal action when violative residues of the sponsored compound occur.

To provide the necessary degree of discrimination, the regulations require that the assay be capable of producing a response when the marker residue is present in target tissue at or above R_m that is, with 99 percent confidence, different from the response in nontreated (control) target tissue (i.e., the difference between the responses of control target tissue and target tissue containing the marker residue in target tissues at or above R_m is, with 99 percent confidence, greater than zero). The actual lowest limit of reliable measurement, L_m, will be determined by reference to the analytical curve of the proposed assay. If the determined lowest limit of reliable measurement, L_m, of the proposed assay is at or below the R_m as determined in accordance with paragraph VI.B.3. or paragraph VII.E. of this preamble, this criterion shall be considered satisfied. This procedure tests the critical factor of assay precision. Thus, an assay that satisfies this criterion will provide a reliable regulatory tool to enable the Commissioner to discriminate safe from unsafe food.

An assay that satisfies this criterion will often have a high signal-noise ratio, although this ratio may be a function of the fluctuations in the equipment used to conduct the assay. The mechanism established by the regulations is geared to the assay's variability; if the assay yields readily reproducible results, the importance of determining the signal-noise ratio is diminished. Every regulation has a zone of ambiguity, however, and the Commissioner believes that it is not now appropriate to define more precisely this requirement for an assay's approvability. In such instances, the professional judgment of the reviewing scientist will come in to play within prescribed limits. Sophisticated methods of statistically analyzing the results of assays offer the promise of more refined standards for this criterion that will take into account assay variation and yet yield the high degree of confidence in assay results, e.g., regression analysis of the spiked tissue, dosed tissue, and tissue blank results. The agency, in conjunction with the Animal and Plant Health Inspection Service of the U.S. Department of Agriculture (APHIS), will be developing in guidelines for further refining this criterion and may subsequently propose amendment of the regulations to prescribe precise standards for evaluating assay accuracy.

The Commissioner recognizes that the term "method sensitivity" is widely used to describe the lowest level of a compound under analysis which can be detected as measured with an analytical assay. Indeed, the original proposal used this term to describe what in the final order has been termed "the lowest limit of reliable measurement." However, there is some confusion surrounding the term "sensitivity," which derives in part from the fact that the term has been

used in two senses: (1) As the lowest level of a compound which can be detected by an assay; and (2) as the lowest level of a compound which can be measured reliably by an assay. In fact, the correct meaning of the term "method sensitivity" is unrelated to a particular level of compound concentration, but rather relates to the ratio of change in instrument response to the change in compound concentration. The term "sensitivity" has therefore been dropped from the final regulations. The Commissioner has adopted the term "lowest level of reliable measurement" because that term more precisely describes the attribute.

In response to comments urging that any "detected residue" should be subject to regulatory control, the Commissioner points out that it is an inherent characteristic of almost all analytical methods that compounds can sometimes be detected at levels below the levels at which they can be reliably measured. More precisely, detection of a compound simply means that there is some instrument response above background levels which could be the compound of interest, but this response cannot be considered as a reliable measurement of the compound. Since protection of public health is the issue, the Commissioner must be in a position to document conclusions based on analytical data, often in a court of law. A major aim of these regulations is to assure that assays used to obtain such data can reliably measure residues. Hence, the Commissioner concludes that the discriminant for samples containing potentially violative exogenous marker residues shall be the lowest limit of reliable measurement, L_m, of the approved assay. Moreover, by imposing these criteria at the preapproval stage, the Commissioner will provide an added measure of public health protection by barring potentially unsafe compounds from the market place.

F. INTERLABORATORY VALIDATION OF ASSAY

Although FDA will review the assays for each sponsored compound, the actual regulatory field screening of foods of animal origin will be primarily performed by APHIS, pursuant to the Meat and Poultry Products Inspection Acts, and by the States pursuant to the Public Health Service Act. The Food and Drug Administration performs a complementary regualtory function: followup analytical and field investigations of violative residues to assemble evidence for use in regulatory actions.

The initial paper review by FDA of material in a petittion permits the agency to make a threshold determination of the acceptability of an assay. Adequate protection of the public health, however, requires assurance that these assays will function in the Government's regulatory laboratories. Therefore, these regulations also prescribe the procedure that will be used to assure that an assay is appropriate for use as a regulatory tool by Government laboratories.

The Commissioner will require that three Government laboratories (two FDA facilities and one USDA facility) independently validate an assay before he can determine that use of a sponsored compound can be approved. The delicate nature of the assays, their importance in assuring that no residues of carcinogenic concern will occur in food of animal origin, and the practical limitations on the Government's capacity to monitor food production and distribution make this requirement mandatory. These three laboratories must study an assay sufficiently to assure that the conclusions about its acceptability drawn by the petitioner in his submission are correct and that all criteria are met.

G. CONCLUSION

If an assay complies with the criteria described above and prescribed by the final regulations, and compliance can be verified under actual conditions of regulatory use, the Commissioner will approve the assay. A full description of the approved assay will be published in the FEDERAL REGISTER upon approval of the petition, in accordance with the provisos to the anticancer clauses and section 512(i) of the act.

IX. WITHDRAWAL PERIODS

A. INTRODUCTION

The regulations define the withdrawal period for a sponsored compound as the time required, after cessation of target animal exposure to the sponsored compound, for the marker residue to deplete to L_m in the target tissue. The withdrawal period must also be compatible with actual conditions of livestock management and reasonably certain to be followed in practice. Because of the way in which the regulations define marker residue, target tissue, and L_m, the use of a sponsored compound in accordance with the prescribed withdrawal period will assure that no carcinogenic residues of such compound will be present in human food derived from treated animals. At any point after cessation of exposure but prior to the determined withdrawal period, treated animal tissues must be considered as containing residues of carcinogenic concern. Thus, the withdrawal period specifies the length of time after the last treatment with a sponsored compound in which animals shall not be slaughtered for food and during which milk shall be discarded.

Several comments addressed the procedures for establishing posttreatment withdrawal periods. Some contended that the requirement for tissue equilibration with residues in the experimental procedure for establishing withdrawal times was inappropriate for therapeutic drugs. Other comments suggested that the withdrawal periods be established to assure the absence of residues from edible tissues only, since they are the ones destined for human consumption. Finally, some comments expressed concern about the practicality of applying confidence-interval techniques to establishing withdrawal periods, especially when dealing with large animals.

B. DATA TO SUPPORT WITHDRAWAL PERIODS

The depletion studies required by the regulations to establish withdrawal periods must take into account the biological variability among animals and other variables that may influence depletion times.

Residue depletion studies must be conducted under conditions of the sponsored compound's maximum proposed use. If a petitioner can demonstrate target tissue equilibration with the marker residue, however, a shorter period of administration of the maximum dose can be permitted. The conditions of the study must also simulate actual use practice. That a compound is intended for a therapeutic use is irrelevant, because the function of this study is to determine the safe withdrawal period, regardless of the compound's intended mode of use. The proposed regulatory assay must be used to measure the marker residue in the target tissue, including milk and eggs where appropriate, because it is this assay that will be used for regulatory monitoring.

All raw data and evaluations must be submitted with the petition along with a graphical presentation of the tissue depletion curve (concentration of marker residue in target tissue versus time).

The analysis of the data must include the estimated depletion curve, which in most instances can be adequately approximated by a first order decay process. The upper 99 percent confidence bound will be determined for the samples from individual target animals and the time of intersection of this upper 99 percent confidence bound with the L_m value will be determined. The withdrawal period is the interval of time between the last administration of the compound and the time of intersection of the upper 99 percent confidence bound on the observations and the L_m of the approved regulatory assay, plus an additional interval determined by rounding out this time interval to provide a practical withdrawal period compatible with animal management practices.

For example, if the time of intersection of the upper 99 percent confidence bound on the individual tissue determinations and the L_m for the marker residue is 39 hours, the withdrawal period (preslaughter interval) would be established as 2 days. In the case of milk samples, if the time of intersection were 63 hours, a withdrawal time of 72 hours (discard of 6 milkings) would be established.

The use of a compound could not be approved if the necessary withdrawal period exceeds a period that is compatible with animal management practices. For example, the use of a compound in lactating animals will not be approved if the required withdrawal time for milk exceeds 96 hours (4 days) because the economics of milk production make observance of such discard times unlikely, or at least not reasonably certain, to be followed in practice.

When the marker residue is an endogenous compound, the withdrawal period is the time after cessation of ad-

ministration of the sponsored compound required for the norm to be restored, with 99 percent assurance, extended if necessary to be compatible with conditions of livestock management. The validated regulatory assay must be used to collect this information.

C RATIONALE FOR USING THE CONFIDENCE BOUND APPROACH

To establish that carcinogenic residues are absent from edible tissues of food-producing animals treated with the sponsored compound, the Commissioner must have information about the rate of residue depletion and the inherent metabolic variabilities among individual target animals. Confidence bounds on experimental data are the only means to allow prediction, with a given degree of confidence, of what will occur in the total target animal population. The Commissioner has prescribed 99 percent confidence bounds throughout these regulations as the degree of confidence necessary to assure protection of public health.

X. WAIVER OF REQUIREMENTS

The regulations permit the Commissioner, in response to a petitioner's request or on his own initiative, to waive, in whole or in part, any of the foregoing requirements for the scientific evaluation of sponsored compounds that have the potential to contaminate human food with residues whose consumption could engender a human risk of carcinogenesis. When an agency particularizes a statutory standard of conduct by regulation, due process requires that it permit affected parties to demonstrate how their alternative mechanism satisfies the statutory standard, and why the regulation should then be waived in the public interest. "Weinberger v. Hynson, Westcott, and Dunning, Inc.," 412 U.S. 609, 620 (1973). Moreover, it has been long settled that an agency may adopt a rule shown to be appropriate for the generality of instances and leave the correction of injustices to applications by those concerned. "National Nutritional Foods Ass'n v. Food and Drug Administration," 504 F.2d 761, 784 (2d Cir. 1974). For these reasons, the Commissioner has expressly included the waiver provision. The Commissioner advises, however, that a waiver will be granted only in exceptional circumstances, and, as the regulation provides, the basis for any waiver must be extensively documented.

XI. IMPLEMENTATION

The proposal would have applied the requirements of the regulations to all new approvals (basic or supplemental) filed or approved after the effective date of the regulations. Prior approvals were to be dealt with on a class-by-class basis, beginning with known carcinogens, suspect carcinogens, and continuing through all compounds previously approved on the basis of zero tolerance. These were to be reviewed as part of the agency's general safety review for previously approved new animal drugs.

The final regulations apply to all new animal drug applications, feed additive petitions, and appropriate color additive petitions, including appropriate supplemental applications, submitted subsequent to the effective date of the regulations. In addition, the requirements of the regulations shall apply to all pending petitions and applications unless the Commissioner determines that compliance with the anticancer provisions of the act can be adequately assured by requiring completion of one or more of the required studies subsequent to approval. The criteria set forth in the regulations are based on generally recognized scientific principles for testing and evaluating chemical compounds for potential carcinogenesis requirements that Congress contemplated FDA would adhere to when it enacted the Food Additives Amendment of 1958 and the Animal Drug Amendments of 1968 (21 U.S.C. 348 (b) and (c) and 360b (b) and (d)).

The Food and Drug Administration has already applied these standards to compounds currently being evaluated for approval or subject to proposals to withdraw approval (e.g. diethylstilbestrol published in the FEDERAL REGISTER of November 2, 1976 (41 FR 52105) and the nitrofurans published in the FEDERAL REGISTER of May 13, 1976 (41 FR 19906) and August 17, 1976 (41 FR 34884)). Accordingly, all previously approved applications for compounds subject to the anticancer clauses will be reviewed as part of the general review of the safety of marketed animal drugs. When the agency finds deficiencies in the data supporting a prior approval, it will issue either a FEDERAL REGISTER notice or a letter pursuant to section 512(l)(1) of the act establishing the time within which the provisions of these regulations must be satisfied. For notices previously published or letters previously issued, the criteria of these regulations will be used to determine whether the data supporting applications are acceptable. The Commissioner will, however, immediately proceed to withdraw approval of applications on the basis of information indicating that a health hazard exists or that no studies necessary to bring a sponsored compound into compliance with the regulation have been conducted.

ADDITIONAL TIME FOR COMMENT

These final regulations largely reflect not only the proposal published in July 1973, but the current FDA practice in reviewing sponsored compounds. Comments on the proposal and petitions filed during the intervening 3 years have raised most of the issues discussed in this preamble and resolved in the final regulations. In the main, therefore, the regulations embody no new decisions. The DES proviso to the anticancer clauses is self-executing, and FDA has therefore been obligated to deal with the issues posed by carcinogenic compounds proposed for use in food-producing animals in the absense of regulations. Accordingly, the Commissioner concludes that these regulations shall become effective March 23, 1977.

Nevertheless, the Commissioner recognizes that it has been over 3 years since these regulations were proposed and that the final regulations resolve some issues not specifically dealt with in the proposal but raised by the comments. For these reasons, the Commissioner is providing an additional 60 days for any interested person to submit further comments on these specific issues. The Commissioner will evaluate any additional comments and will later publish any revisions to the final regulations, if appropriate.

The Commissioner urges that any comments submitted within this additional period address only new issues, and not reopen matters raised by the initial proposal and discussed in this preamble. The Commissioner is particularly interested in receiving comments on four specific areas of the regulations. First, he invites further discussion of the acceptable level of risk for use in the modified Mantel-Bryan calculation. At the present time, FDA is involved in administrative adjudications concerning potentially carcinogenic animal drugs. These proceedings may assemble additional evidence on the acceptable level of risk. Because this issue is important to application of the regulations, the Commissioner believes additional comment will contribute to public understanding. This action will in no way jeopardize the public health, for the administrative record adequately supports the current level of risk; the Commissioner is interested in comments on whether the level of risk should be further reduced.

Second, the Commissioner will entertain comments on the concept of comparative metabolism. This unique approach was developed in response to the diverse comments on the issue of which metabolites of a sponsored compound, if any, should be tested. An analogous procedure of the Environmental Protection Agency has received judicial approval. "Environmental Defense Fund, Inc., et al., v. Environmental Protection Agency," No. 75-2259, (D.C. Cir., November 10, 1976), slip op. at 14. The Commissioner welcomes suggestions for alternatives to this approach.

Third, as previously noted, the Commissioner invited suggestions for alternative mechanisms for dealing with endogenous compounds. Several comments on the proposal urged that an alternative procedure for evaluation of such compounds should be available, but failed to suggest any feasible approaches.

Finally, the Commissioner welcomes suggestions of refined mechanisms for statistically differentiating target tissue containing the market residue from blank target tissue.

The Commissioner concludes that all of the provisions of the final regulations should be implemented pending reconsideration of any specific provisions based upon additional comments. This will work no hardship since all provisions of the regulations are supported by the record, and, except for the level of risk, the only changes the Commissioner contemplates concern alternative methods of satisfying the statutory requirements.

The Commissioner has carefully considered the environmental effects of the regulations and, because this action will not significantly affect the quality of the

human environment, has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20852.

This final order was proposed prior to Executive Order 11821, requiring agencies in the executive branch to review regulatory and legislative proposals they initiate for inflation impact, and so does not require inflation impact review.

Therefore, under the Federal Food, Drug, and Cosmetic Act (sections 402, 403, 409, 512, 701(a), 706, 52 Stat. 1046–1048 as amended, 1055, 72 Stat. 1785–1788 as amended, 74 Stat. 399–403, 82 Stat. 343–351 (21 U.S.C. 342, 343, 348, 360b, 371(a), 376)) and under authority delegated to the Commissioner (21 CFR 5.1) (recodification published in the FEDERAL REGISTER of June 15, 1976 (41 FR 24262)), Chapter I of Title 21 of the Code of Federal Regulations is amended as follows:

PART 8—COLOR ADDITIVES

1. In Part 8, by amending § 8.36 by adding new paragraph (c) to read as follows:

§ 8.36 Application of the cancer clause of section 706 of the act.

* * * * *

(c) *Color additives for use as an ingredient of feed for animals that are raised for food production.* Color additives that are an ingredient of the feed for animals that are raised for food production must satisfy the requirements imposed by subpart E of Part 500 of this chapter.

PART 500—GENERAL

2. In Part 500, by adding a new Subpart E, consisting of §§ 500.80 through 500.98, to read as follows:

Subpart E—Criteria and Procedures for Evaluating Assays for Carcinogenic Residues in Edible Products of Animals

Secs.

500.80 Chemical compounds used in food-producing animals; procedures and criteria for determining the acceptability of assay methods for carcinogenic residues in edible products of such animals.
500.84 Metabolic study in target animals to identify residues for chronic testing.
500.85 Criteria for test animal selection; comparative metabolic studies to aid in assessing the carcinogenicity of residues that cannot practicably be tested individually (intractable residues).
500.87 Chronic testing.
500.89 Metabolic study to identify the marker residue and target tissue.
500.90 Evaluation and approval of a regulatory assay.
500.92 Withdrawal periods.
500.94 Publication of the approved regulatory assay.
500.96 Waiver of requirements.
500.98 Implementation.

AUTHORITY: Secs. 402, 408, 409, 512, 701(a), 706, 52 Stat. 1046–1048 as amended, 1055, 72 Stat. 1785–1788 as amended, 74 Stat. 399–403, 82 Stat. 343–351 (21 U.S.C. 342, 343, 348, 360b, 371(a), 376).

Subpart E—Criteria and Procedures for Evaluating Assays for Carcinogenic Residues in Edible Products of Animals

§ 500.80 Chemical compounds used in food-producing animals; procedures and criteria for determining the acceptability of assay methods for carcinogenic residues in edible products of such animals.

(a) *Purpose and applicability of this subpart.* (1) The act requires that compounds intended for use in food-producing animals shall be safe and that food produced from animals exposed to such compounds be safe for human consumption, and prohibits the use of any compound found to induce cancer when ingested by man or animal in food-producing animals unless it can be determined by methods of examination prescribed or approved by the Commissioner that no residue of such compound will be found in the food produced from such animals under conditions of use reasonably certain to be followed in practice. Petitions for the approval of the use of a compound in food-producing animals shall include adequate data for establishing the absence of residues of carcinogenic compounds in the food produced from such animals.

(2) The provisions of this subpart establish the following: (i) The lowest limit of reliable measurement for the regulatory assay required for carcinogenic residues by sections 409(c)(3)(A), 512(d)(1)(H), and 706(b)(5)(B) and sections 409(b)(2)(D), 512(b)(7) and 706(b)(5)(A)(iv) of the act;

(ii) The procedures and criteria for evaluation and approving such assays; and

(iii) The procedures and criteria for establishing the premarketing withdrawal period for use of compounds likely to produce such residues.

(3) This subpart shall apply specifically to compounds intended for use in food-producing animals and their feed that have the potential to contaminate human food with residues whose consumption could engender a human risk of carcinogenesis. The determination of this potential shall be based on considerations of chemical, biochemical, physiological, and toxicological data derived from the scientific literature and from other sources available to the sponsor or to the Commissioner and on the proposed patterns of compound use. The subpart establishes a sequential process for the collection of other chemical, biochemical, physiological, and toxicological data pertinent to the safety of the proposed use of the sponsored compound. This subpart shall not apply to essential nutrients.

(b) *General approach.* (1) When the Commissioner determine that a sponsored compound has the potential to contaminate food from food-producing animals with residues (the sponsored compound, metabolites, conversion products, or any other substances formed in or on food because of the compound's use) whose consumption may engender a human risk of carcinogenesis, the following procedure for data collection and evaluation shall become applicable:

(i) A metabolic study in the animals in which the sponsored compound is intended for use (target animals) designed to identify metabolites of concern;

(ii) A metabolic study of the sponsored compound in experimental animals designed to aid in assessing the carcinogenicity of residues that cannot practicably be tested individually (intractable residues);

(iii) Chronic testing in test animals to assess the carcinogenic potential of residues of the sponsored compound and to furnish data suitable for statistical treatment by the procedure of Mantel and Bryan, (Mantel, N., and W. R. Bryan, "'Safety' Testing of Carcinogenic Agents," "Journal of the National Cancer Institute," 27(2):455–470 (1971)) as modified by Mantel et al. (Mantel, N., et al., "Improved Mantel-Bryan Procedure for 'Safety' Testing of Carcinogens," "Cancer Research," 35:865–872 (1975)) [3] and by this subpart, to permit the no-residue requirement of the act to be operationally defined for purposes of establishing a lowest limit of reliable measurement for an assay to measure residues of the sponsored compound;

(iv) A detailed metabolic study of the sponsored compound in target animals designed to identify a specific residue and tissue to serve as indicators (marker residue and target tissue) to determine whether the no-residue requirement of the act is satisfied;

(v) Development of a regulatory assay to measure the marker residue in the target tissue at and above the level operationally defined as satisfying the no-residue requirement of the act; and

(vi) Establishment of the premarketing withdrawal period required for the safe use of the sponsored compound.

(2) If, at any point in the sequential process of data collection set forth in paragraph (b)(1) of this section, the evaluation of the data satisfies the Commissioner that no human risk of carcinogenesis attaches to the proposed use of the sponsored compound, the compound shall be considered for approval under the general safety provisions of the act.

§ 500.84 Metabolic study in target animals to identify residues for chronic testing.

(a) A metabolic study, described in paragraph (b) of this section, shall be conducted in target animals to provide data on the physicochemical characteristics of residues, their relative proportions, their distribution among the various edible tissues (which include milk or eggs when applicable), and their retention and depletion by the animals.

(b) The target animal metabolic study shall satisfy the following minimum requirements:

(1) The metabolic study shall be conducted in target animals with the spon-

[3] Copies may be obtained from: Associate Director for Scientific Evaluation (HFV–100), Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

sored compound bearing appropriate radiolabels, unless other expermental methods permit equivalent measurement of residues. Such labels must assure that residues containing structural moieties of potential carcinogenic concern can be detected and measured in edible tissues at levels as low as the best available technology will permit. Hypotheses about the sponsored compound's projected metabolic pathways may be used as a guide to experimentation, but shall not be a substitute for actual experimentation.

(2) The dosing regimen shall be consistent with the maximum proposed use level and proposed duration of exposure to the sponsored compound. For a compound that is proposed for continuous or repeated use in target animals, administration for the metabolic study need continue only until residue equilibration or tissue saturation has been demonstrated.

(3) The metabolic study shall be designed to yield the following information:

(i) The concentrations and total number of residues detected in edible tissues of target animals immediately following cessation of exposure;

(ii) Except when the Commissioner specifies otherwise, the concentrations and total number of residues detected in edible tissues of target animals when the total residue burden has depleted for at least three half-lives; and

(iii) The physicochemical properties of the detected residues to identify compounds of potential carcinogenic concern.

(4) The results of the metabolic study shall be submitted in the form of a detailed report conforming to the standards required of scientific manuscripts submitted for publication in the journals of professional scientific societies such as the American Chemical Society and the American Society of Biological Chemists. In addition, all raw data shall accompany and be referenced in the report.

(c) If the Commissioner determines that a sponsored compound has potential to contaminate food with residues whose consumption engenders human risk of carcinogenesis, the petitioner shall be required to determine the carcinogenic potency of the sponsored compound and any of its residues that might be of public health concern because of chemical structure or persistence and concentration in edible tissues. Ordinarily, chronic testing of the sponsored compound and selected residues in experimental animals shall be the preferred means of assessing carcinogenic potency. (Section 500.85 describes an alternative means of assessing the carcinogenic potency of residues whose isolation or synthesis in sufficient quantities for chronic testing proves to be beyond the practical limits of current chemical technology (intractable residues) by establishing additional criteria for selecting test animal species/strains used to conduct chronic toxicity testing of the sponsored compound.)

§ 500.85 Criteria for test animal selection; comparative metabolic studies to aid in assessing the carcinogenicity of residues that cannot practicably be tested individually (intractable residues).

(a) The primary criterion for the selection of species or strains of test animals for chronic testing of the sponsored compound and any metabolites selected in accordance with § 500.84 shall be the suitability of the species or strain as a model for man.

(b) If one or more intractable residues are also selected for chronic testing based upon the metabolic study in the target animal, a secondary criterion for the selection of species or strains of animals for the testing of the sponsored compound shall be employed. Metabolic studies of the sponsored compound in the test animal species or strains deemed suitable for chronic testing by the primary criterion shall be conducted to determine if the intractable residues present in the tissues of target animals are also produced in the test animals. Chronic testing of the sponsored compound in a species or strain of test animals in which the residues produced are similar to the complement of residues in the tissues of the target animals shall be considered an appropriate method of assessing the carcinogenic potency of the intractable residues.

§ 500.87 Chronic testing.

(a) Chronic toxicity tests shall be conducted to assess the carcinogenic potential of the residues of the sponsored compound.

(1) The sponsored compound and any residues selected for chronic toxicity testing shall be subjected to oral, lifetime, dose-response studies in the test animal species or strains selected in accordance with § 500.85. Each of these studies must be designed to determine whether the test compound is carcinogenic. Protocols for these studies should be submitted to the Commissioner for review prior to commencing testing.

(2) The Commissioner will determine whether any of the compounds tested is carcinogenic on the basis of the results of these chronic toxicity studies and other available information. If this evidence is equivocal, the compound shall be classed as a carcinogen until further testing resolves any remaining questions regarding carcinogenicity.

(b) When the Commissioner determines that a sponsored compound has the potential to increase the normal levels (pools) of carcinogenic and potentially carcinogenic substances endogenous to the target animals, the petitioner shall meet the requirements of § 500.89 (c), (d) and (e).

(c) For each tested compound classed as a carcinogen, the appropriate data from the chronic dose-response studies shall be analyzed according to procedures described by Mantel and Bryan (Mantel, N., and W. R. Bryan, " 'Safety' Testing of Carcinogenic Agents," "Journal of the National Cancer Institute," 27(2):455–470 (1961)) and Mantel et al. (Mantel, N., et al., "Improved Mantel-Bryan Procedure for 'Safety' Testing of Carcinogens," "Cancer Research," 35:865–872 (1975))[8]; subject to the modifications and restrictions set forth in paragraph (c)(1) through (9) of this section. The purpose of this analysis shall be to define the no-residue requirement of the act as it applies to the total residue of carcinogenic concern of the sponsored compound and thereby to determine the lowest level of reliable measurement that shall be required for a regulatory assay to be approved for the measurement of such residues.

(1) The administered dose of each test compound shall be expressed as a fraction of the total diet fed the test animal species/strains, e.g., parts per million, parts per billion, etc.

(2) The "safe" level of Mantel and Bryan, calculated for each test compound in accordance with this section, shall be expressed as a fraction of the total diet fed the test animal species/strains. It shall be calculated with 99 percent confidence for a maximum lifetime risk that is essentially zero but never expected to exceed 1 in 1 million.

(3) A slope of one probit per unit log dose shall be used for extrapolation to the "safe" level unless the experimental data indicate that a shallower slope is required to maintain the conservatism of the procedure.

(4) Data obtained from more than one dose level fed to groups of experimental animals of the same strain shall be combined as described by Mantel et al. (Mantel, N., et al., "Improved Mantel-Bryan Procedure for 'Safety' Testing of Carcinogen," "Cancer Research", 35:865–872 (1975)),[8] and subject to the restrictions specified by these authors.

(5) Pooling data from various chronic tests using different animal sexes, species, or strains shall be permitted if it can be demonstrated that the protocols are of compatible design. If statistically significant biological differences in tumorigenic responses are observed between sexes or among species or strains of experimental animals, only subsets of data representing statistically and biologically compatible bioassays may be combined for analysis.

(6) All tumors (benign and/or malignant) shall be considered in the analysis.

(7) The number of animals at risk may be adjusted for competing risks unrelated to the compound-induced carcinogenesis only when the data clearly support such an adjustment.

(8) When only the sponsored compound is subjected to chronic testing, the calculated "safe" level shall be designated at S_e. When more than one compound is subjected to chronic testing, the

lowest of all calculated "safe" levels shall be designated S_s. S_s shall be expressed as the fraction of the diet fed the test animals (e.g., parts per million, parts per billion, etc.).

(9) The no-residue requirement of the the act shall be considered satisfied when conditions and use of the compound, including any required withdrawal period, can be prescribed to assure that the sum of the levels of all potential residues of carcinogenic concern will not exceed S_s in the total diet of man and a regulatory assay is available that is capable of reliably measuring such residues at and above that level. All residues of the sponsored compound shall be classed as carcinogenic except those that have been unequivocally shown to be noncarcinogenic.

(d) The S_s value represents the sum of all residues of carcinogenic concern that must not be exceeded in the total diet of man. For individual edible tissues, the value that must not be exceeded shall be designated S_m and calculated according to the following formula:

$$S_m = \frac{S_s}{T}$$

NOTE.—T is the fraction of the total daily diet of man represented by an individual edible tissue.

(1) The principal S_m calculations are as follows:

Edible tissue	T	S_m
Muscle	1/3	$3S_s$
Milk	1	S_s
Eggs	1/3	$3S_s$

(2) Calculation of S_m for tissues consumed less frequently than muscle may take into consideration the frequency of consumption of such tissues if it can be clearly shown that S_s will not be exceeded in the total human diet.

§ 500.89 Metabolic study to identify the marker residue and target tissue.

(a) The petitioner shall conduct a study of the metabolic fate of the sponsored compound in target animals adequate to provide the data necessary for the selection of a marker residue in target tissue.

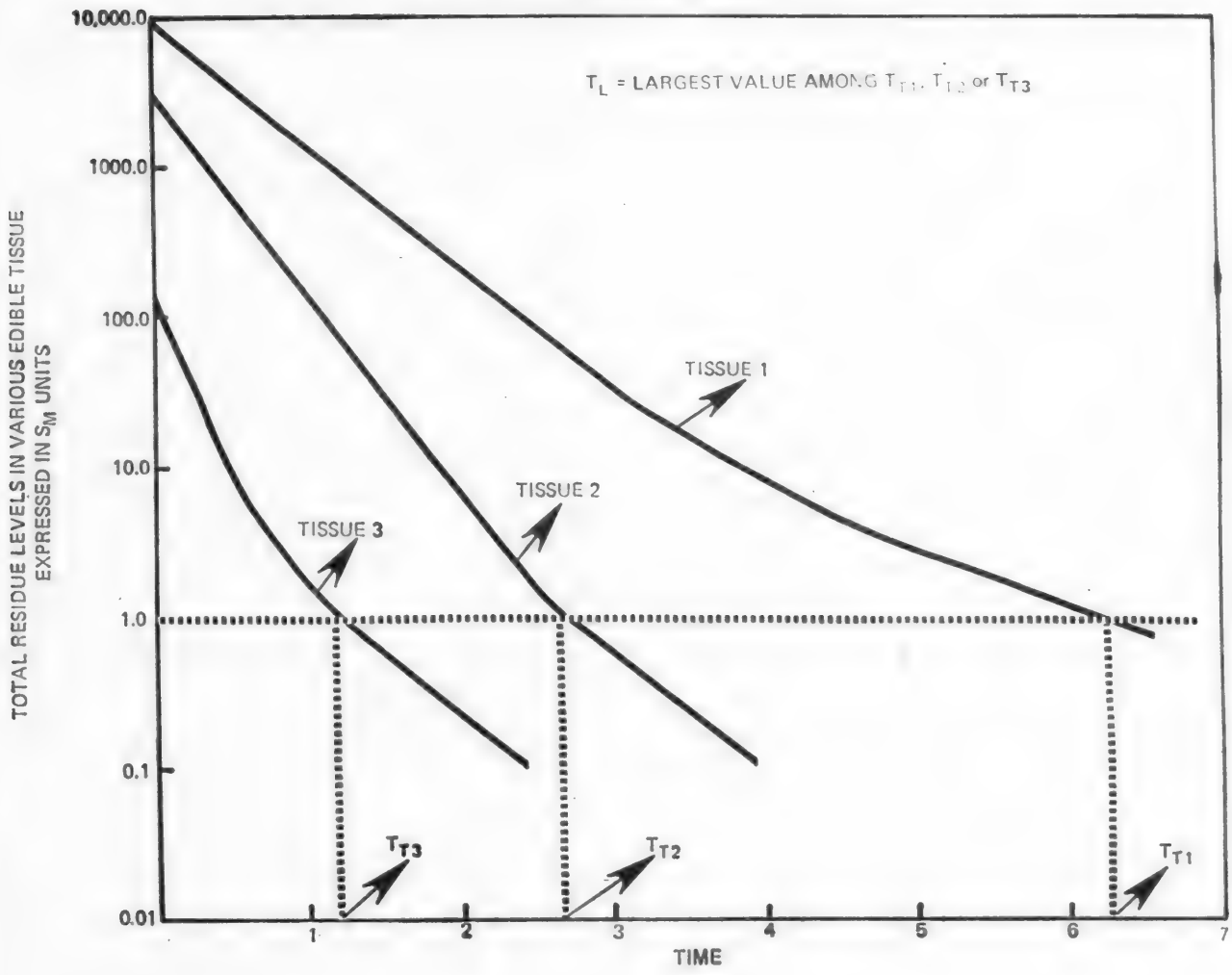
(1) The target tissue is that tissue in which measurement of the total residue burden of carcinogenic concern is a reliable measure of the total residue burden of carcinogenic concern in all edible tissues.

(2) The marker residue for the sponsored compound shall be that residue (the sponsored compound, any metabolite, or more than one of these) whose level in the target tissue is a reliable measure of the total burden of all residues of carcinogenic concern in all edible tissues.

(b) The metabolic study to establish the marker residue and target tissue shall comply with the requirements set forth in § 500.84(b) (2) and (4), with the following additional specifications:

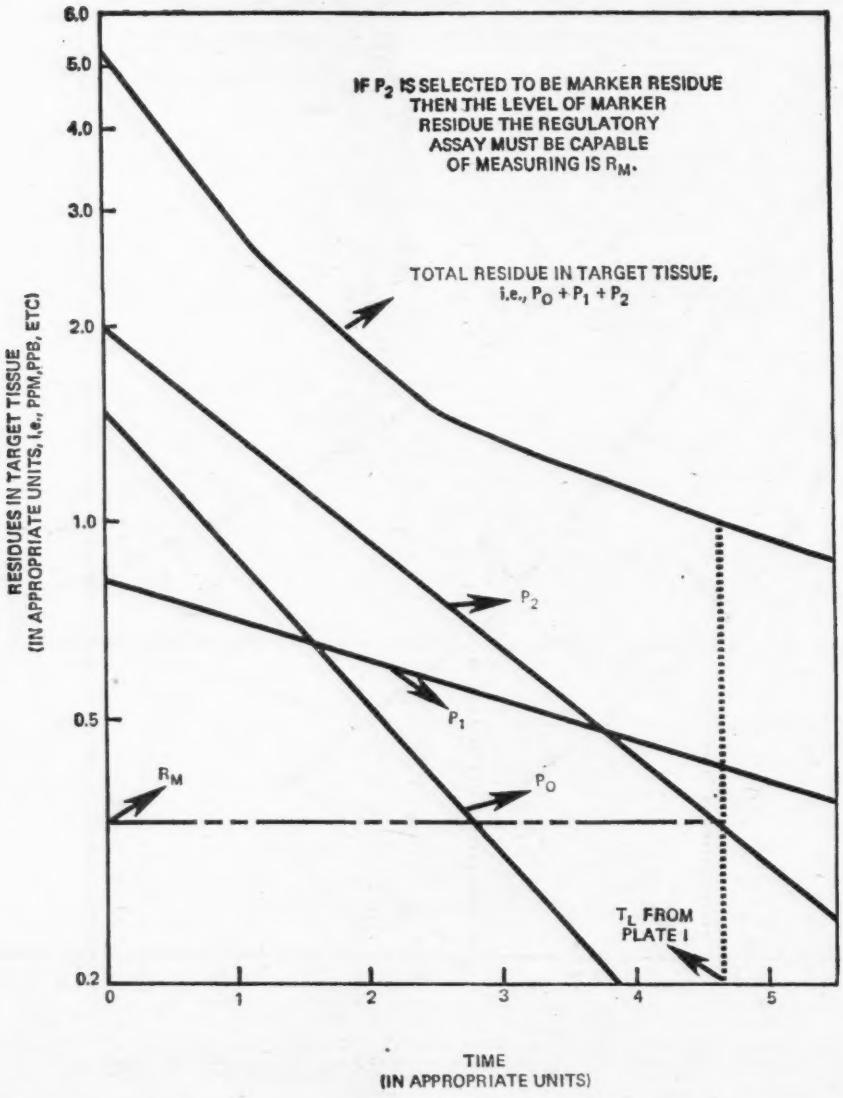
(1) For each edible tissue, the depletion profile of the total residue of carcinogenic concern shall be constructed and shall include measurements of levels at least as low as the S_m appropriate to the tissue under study, set forth in Plate I as follows:

PLATE I. RESIDUE DEPLETION CURVES TO BE USED IN THE DETERMINATION OF MARKER RESIDUE AND TARGET TISSUE.



(2) Depletion profiles for one or more potential marker residues shall be constructed as set forth in Plate II in this paragraph, and shall include measurements of levels corresponding to the time when the total residue level has reached S_M in the edible tissue requiring the longest time to deplete to S_M (T_L of Plate I in paragraph (b) (1) of this section).

PLATE II. SELECTION OF MARKER RESIDUE AND ITS LEVEL R_M THAT MUST BE MEASURED BY THE REGULATORY ASSAY.



(3) If these specifications have been met by the metabolic study required by § 500.84(b), a second metabolic study need not be performed to satisfy this section.

(4) From these data, the Commissioner will select a marker residue and target tissue, and he will also designate the required level of marker residue, R_m (set forth in Plate II in paragraph (b)(2) of this section), that regulatory assays must be capable of measuring in the target tissue. The selection of R_m shall be such that the absence of the marker residue in target tissue above R_m can be taken as confirmation that the total residue burden of carcinogenic concern does not exceed S_m in each of the various edible tissues and therefore that the total burden of carcinogenic concern in the human diet does not exceed S_o.

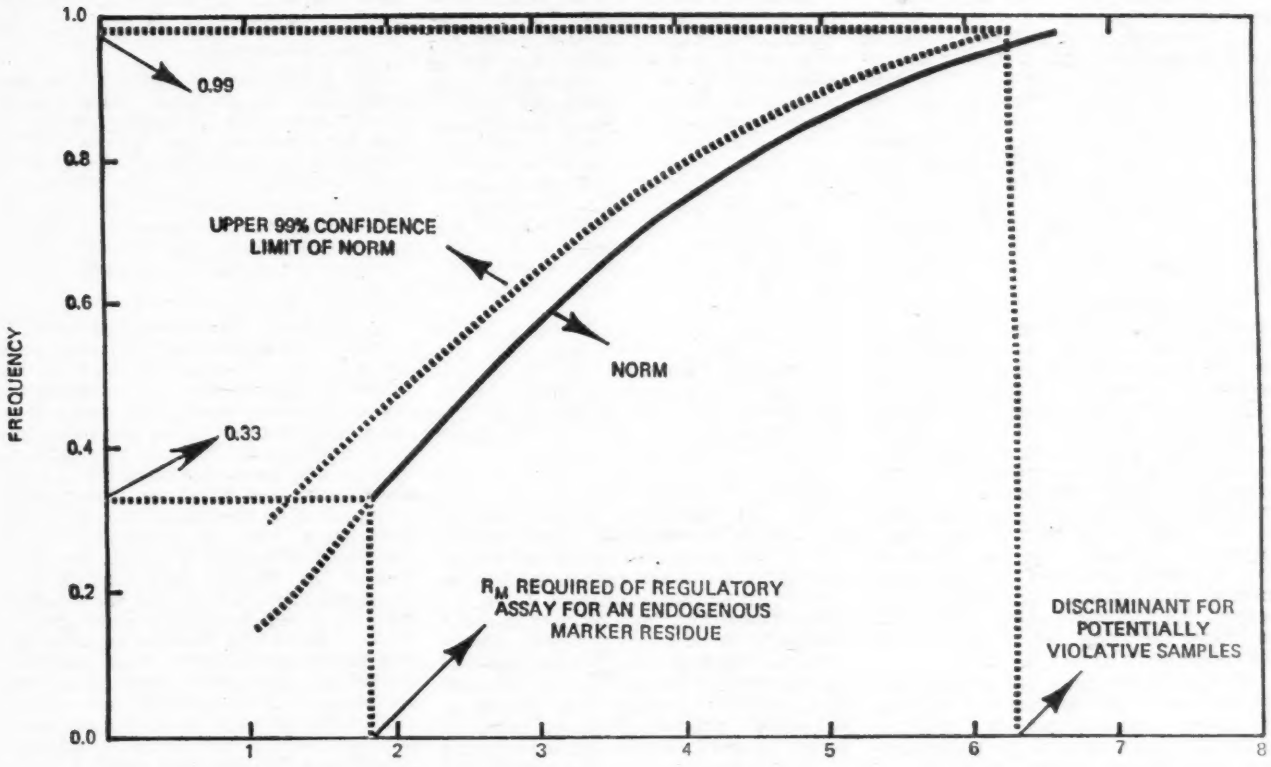
(c) When the Commissioner determines on the basis of available scientific information that a sponsored compound has the potential to increase the normal levels (pools) of potentially carcinogenic substances endogenous to target animals, the petitioner shall provide the following additional data:

(1) An experimental determination of the background levels (norm) of each of the potentially carcinogenic endogenous substances of concern in untreated target animals.

(i) The norm shall be specific for the target animals and the intended conditions of animal husbandry, and shall be determined from studies designed to take into account differences due to factors such as breed, age, sex, state of estrus, and geographic location.

(ii) Each norm shall be submitted in the form of a graph of the cumulative frequency distribution versus the observed naturally occurring levels, including the 99 percent confidence bounds, set forth in Plate III as follows:

PLATE III. SAMPLE OF A NORM



(iii) An assay shall be acceptable for the determination of a norm only if it yields values for the endogenous compound of interest greater than zero in at least two-thirds of the untreated target animals.

(2) Studies to measure the effect of the sponsored compound on the norm and the postexposure decay of any increase in the norm caused by administration of the sponsored compound.

(3) All data from these studies submitted in accordance with the requirements established in paragraph § 500.-84(b)(4).

(d) For a potentially carcinogenic endogenous compound whose norm is increased by the administration of a sponsored compound, the no-residue requirement of the act shall be considered satisfied when the norm is restored.

(1) The norm shall be considered restored when the distribution of values for the endogenous substance of concern observed in a group of treated animals is with 99 percent confidence the same as the norm.

(2) The marker residue for a sponsored compound that affects a potentially carcinogenic endogenous substance shall be the affected endogenous substance.

(3) When the norm of more than one potentially carcinogenic endogenous compound is increased by administration of the sponsored compound, the marker residue for all endogenous compounds of concern shall be that endogenous compound whose norm requires the longest time for restoration.

(e) For an endogenous compound selected to be a marker residue, the required level of measurement, R_m, for the regulatory assay shall be the level of that endogenous compound corresponding to the 33d percentile of the norm, set forth in Plate III in paragraph (c)(1)(ii) of this section.

§ 500.90 Evaluation and approval of a regulatory assay.

(a) Before a petition can be considered for approval, the petitioner shall submit for evaluation and validation a regulatory assay developed to monitor compliance with no-residue requirement of the act. The regulatory assay shall reliably measure the marker residue in the target tissue at levels at least equal to and above R_m, as defined in § 500.89 (b) and (e). The criteria and procedures in paragraphs (b) through (g) of this section shall apply to the evaluation and approval of assays.

(b) The regulatory assay shall be evaluated and validated using data collected from three types of samples:

(1) Samples containing various known concentrations of marker residue added to the target tissue, i.e., "spiked" tissue samples.

(2) Samples containing various levels of the marker residue obtained from target tissue at appropriate time intervals after the sponsored compound is administered in accordance with the proposed labeling, i.e., "dosed" tissue samples.

(3) Samples obtained from untreated target animals, i.e., "control" tissue samples.

(c) The petition for approval of the proposed regulatory assay shall contain the following:

(1) A complete description of the assay.

(2) A list of all necessary equipment and reagents.

(3) A standard curve prepared from samples of the marker residue of known purity.

(4) An analytical curve of the observed assay response versus the tissue concentrations of the marker residue in spiked target tissue. The curve shall include the 99 percent confidence bounds of a single assay response.

(5) All raw data and worksheets from the analyses of spiked, dosed, and control tissue samples, and from the analysis used in preparing the standard curve, including spectrograms, chromatograms, etc.

(6) A discussion of the data generated in the assay development process pertinent to the evaluation criteria set forth in paragraph (d) of this section explain-

ing how the data show that the proposed assay conforms to those criteria.

(d) A regulatory assay must satisfy the following criteria:

(1) *Dependability.* The assay shall be considered dependable if it does not result in an unreasonable number of failures due to unknown, uncontrollable, or random factors. Evaluation of the data to support the dependability criterion will be based on the total number of assay runs that are started to provide data points for the analytical curve of paragraph (c) (4) of this section. An explanation shall be required for any assay run started that yields no final determination.

(2) *Practicability.* The assay shall be considered practicable only if it is suitable for routine use in a government regulatory laboratory. The time required to complete the assay must be consistent with regulatory objectives (monitoring, compliance, etc.). All supplies, equipment, reagents, standards, and other materials necessary to conduct the assay must be commercially available except that reference standards may be supplied by the petitioner if they are not commercially available. The Commissioner will withdraw approval of any assay method and initiate regulatory action against the sponsored compound, if the petitioner breaches such a condition of the compound's approval.

(3) *Specificity.* The assay shall be considered specific if the observed response is a smooth and continuously decreasing or increasing function of the concentration of the marker residue and that compound only. The regulatory assay must be comprised of a sufficient number of independent measurements based on a different biological, biochemical, or physiochemical principles to assure that the identity of the marker residue is confirmed.

(4) *Accuracy.* The assay shall be considered accurate if the measurements it yields are normally no less than 60 percent nor greater than 110 percent of the marker residue's true concentration in the spiked target tissues.

(5) *Lowest limit of reliable measurement.* The regulatory assay shall be considered approvable if it can reliably discriminate with 99 percent confidence the marker residue response from the target tissue background response at or below the required lowest limit of reliable measurement, the R_m of § 500.89 (b) or (c). If the regulatory assay for an exogenous compound can reliably discriminate the marker residue response from the target tissue background response at a level below the required lowest limit of reliable measurement determined in accordance with § 500.89(b), the Commissioner shall approve the compound for use only under conditions that will not result in residues above that level.

(e) The Commissioner will review and evaluate the data submitted in accordance with paragraphs (a), (b), and (c) of this section. If the Commissioner concludes that the assay satisfies the evaluation criteria of paragraph (d) of this section, it will then be subjected to the interlaboratory validation study described in paragraph (f) of this section.

(f) Two Food and Drug Administration laboratories and one U.S. Department of Agriculture laboratory will independently run a number of assays to ascertain whether the regulatory assay method conforms to the criteria set forth in paragraph (d) of this section.

(1) The petitioner shall supply the validating laboratories with the number and amount of dosed and control tissue samples requested by the Commissioner.

(2) The petitioner shall supply reagents, standards, supplies, and equipment not readily available to the validating laboratories, as requested by the Commissioner.

(g) The Commissioner will evaluate the data gathered from the study described in paragraph (f) of this section. The assay shall be approved if it meets the criteria set forth in paragraph (d) of this section in each of the three validating laboratories.

§ 500.92 Withdrawal periods.

(a) The withdrawal period shall be the time after cessation of administration of the sponsored compound necessary for the marker residue to deplete, with 99 percent assurance, to L_m in the target tissue. The time will be extended if necessary to be consistent with conditions of livestock management reasonably certain to be followed in practice. The petitioner shall submit studies of the marker residue's depletion from the target tissue of animals dosed according to the maximum level of use proposed in the petition. The validated regulatory assay must be used to collect these data.

(1) The petitioner shall submit a plot of the concentration of marker residues in target tissue as a function of time (depletion curve) including the 99 percent confidence limits on the observed values.

(2) All raw data and statistical analyses shall be submitted along with a referenced discussion of the results.

(3) Use of the sponsored compound shall be approved only if the available evidence demonstrates that the proposed conditions of use, including any withdrawal period, are reasonably certain to be followed in practice.

(b) When the marker residue is an endogenous compound, the withdrawal period shall be the time required after cessation of administration of the sponsored compound for the norm to be restored, with 99 percent assurance. The time will be extended if necessary, but not reduced, to be compatible with conditions of livestock management reasonably certain to be followed in practice. The validated regulatory assay must be used to collect data on the rate of restoration of the norm.

(1) The petitioner shall submit a series of curves that demonstrate the time required for restoration of the norm.

(2) All raw data and statistical analysis shall be submitted along with a referenced discussion of the results.

(3) Approval of the petition for the sponsored compound shall be granted only if the available evidence demonstrates that the proposed labeling is reasonably certain to be followed in practice.

§ 500.94 Publication of the approved regulatory assay.

The lowest level of reliable measurement (L_m), the complete regulatory assay for measuring the marker residue in the target tissue, and the analytical curve shall be published in the FEDERAL REGISTER, in accordance with the provisions of sections 409(c)(3)(A), 512 (d)(1)(H) and (I), and 706(b)(5)(B) of the act. For an endogenous marker residue, the norm shall also be published.

§ 500.96 Waiver of requirements.

The Commissioner, in response to a petitioner or on his own initiative, may waive, in whole or in part, any of the foregoing requirements for the scientific evaluation of sponsored compounds that have the potential to contaminate human food with residues whose consumption could engender a human risk of carcinogenesis. A petition for such waiver may be filed by any person who would be adversely affected by the application of the requirements to a particular compound. The petition shall explain and document why some or all of the requirements are not reasonably applicable to the compound, and describe the alternative procedures that have been, or could be, followed to assure that use of the compound will not contaminate human food with residues whose consumption could engender a human risk of carcinogenesis and that an assay method exists that satisfies the requirements of § 500.90(d) (1) through (4) and that is capable of measuring any such residues that might occur when the compound was improperly used. The petition shall set forth clearly the reasons why the alternative procedures will provide the basis for concluding that approval of the compound satisfies the requirements of the anticancer provisions of the act. If the Commissioner determines on his own initiative that waiver of any of the foregoing requirements is appropriate, he shall so state and set forth the basis for that determination in the regulation approving marketing of the sponsored compound.

§ 500.98 Implementation.

(a) The requirements of this subpart shall apply to all new animal drug applications, feed additive petitions, and appropriate color additive petitions (i.e., all compounds intended for use in food-producing animals) submitted to the Food and Drug Administration subsequent to the effective date of the subpart, including appropriate supplemental applications, and to all such applications or petitions on file with the agency on the effective date of the subpart except to the extent that the Commissioner determines that consumer protection can be adequately assured by imposing such requirements in accordance with the provisions of paragraph (b) of this section.

(b) The provisions of this subpart shall also apply to the following compounds already approved:

(1) Those compounds that the Commissioner determines, on the basis of available, reliable information, have been shown to induce cancer when ingested by man or animals.

(2) Those compounds that the Commissioner determines may induce cancer when ingested by man or animals, i.e., suspect carcinogens.

(3) Any compound for which the Commissioner concludes sufficient information has not been provided to determine whether that compound is appropriately regulated under the general food safety provisions of the act or under the anticancer provisions of the act.

(c) Any compound already approved to which the Commissioner determines the anticancer provisions of the act are applicable, or for which additional data are required for such a determination, will be the subject of a notice published in the FEDERAL REGISTER or a letter issued pursuant to section 512(1) of the Act establishing the time within which the requirements of this subpart must be satisfied.

(1) Notices already published in the FEDERAL REGISTER and letters already sent by the Food and Drug Administration requiring additional studies or submission of an improved regulatory assay shall remain in effect, and the provisions of this subpart shall be used in determining compliance with the requirements of the act identified in those notices and letters.

(2) The Commissioner will proceed to withdraw approval of any compound on the basis of data or information indicating a health hazard or in response to any failure to undertake studies necessary to comply with the provisions of this subpart.

PART 514—NEW ANIMAL DRUG APPLICATIONS

3. In Part 514, by amending § 514.111, by adding a new paragraph (a)(10) to read as follows:

§ 514.111 Refusal to approve an application.

(a) * * *

(10) Such drug fails to satisfy the requirements imposed by Subpart E of Part 500 of this chapter.

* * * * *

PART 571—FOOD ADDITIVE PETITIONS

4. In Part 571, by adding a new § 571.115, to read as follows:

§ 571.115 Application of the anticancer cause of section 409I.

Food additives intended for use as an ingredient in food for animals that are raised for food production must satisfy the requirements imposed by Subpart E of Part 500 of this chapter.

Effective date. These regulations shall be effective March 23, 1977. Interested persons may, on or before April 25, 1977 submit to the Hearing Clerk, Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20857, written comments, in quadruplicate and identified with the Hearing Clerk docket number found in brackets on the heading of this document, regarding these regulations. Received comments may be seen in the above office between 9 a.m. and 4 p.m., Monday through Friday. Any changes in this order justified by such comments will be the subject of a further order amending the specific regulations involved.

(Secs. 402, 403, 409, 512, 701(a), 706, 52 Stat. 1046–1048 as amended, 1055, 72 Stat. 1785–1788 as amended, 74 Stat. 399–403, 82 Stat. 343–351 (21 U.S.C. 342, 343, 348, 360b, 371(a), 376).)

NOTE.—Incorporation by reference provisions approved by the Director of the Office of the Federal Register on July 11, 1973, and February 15, 1977, and on file in the library of that office.

Dated: February 14, 1977.

SHERWIN GARDNER,
Acting Commissioner of Food and Drugs.

[FR Doc.77-5266 Filed 2-18-77;8:45 am]